ORIGINAL ARTICLES

ON THE NATURE OF REACTIONS RESPONSIBLE FOR SOIL ACIDITY

PART IV. INTERPRETATION OF TITRATION CURVES

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(Received for publication on 18th October 1935)

(With twenty text-figures)

The work on the titration curves of silicic acid sols [Mukherjee, Roychoudhury, Das Gupta and Chatterjee, 1932; Mukherjee, Roychoudhury, Mukherjee and Majumdar, 1934] has been extended. The method of preparaing the sols was as previously reported [Mukherjee *et al.*, 1932] and as small amounts of hydrochloric acid and sodium chloride are not completely removed by the dialysis, several sols and their ultrafiltrates have been analysed for this purpose. The peculiarities of the titration curves observed previously required further investigation. The curves were often not exactly reproducible and sometimes showed [compare curves I, II, IV, V, VI, for sols I, II, and III; Mukherjee *et al.*, 1932] changes in curvature simulating the steps in the curves of a polybasic acid representing particular stages of its dissociation. Irregularities have also been observed [Sol A and its dilutions; Mukherjee *et al.*, 1934] with sols having visible aggregates suspended in them. In the present paper the titration curves of a series of silicic acid sols obtained with considerably improved experimental arrangements have been given. Different concentrations of alkalis and different alkalis have been compared. The course of interaction between the sol and neutral salts has been followed. The hydrogen electrode gives troubles in extremely dilute unbuffered acid systems and the extent and manner of variations of the e.m.f. have been noted. The pH values obtained from the hydrogen electrode have been compared with corresponding values from the quinhydrone electrode.

EXPERIMENTAL

The sols were prepared and purified as previously reported. The accidental ingress of carbon dioxide, oxygen and impurities from the atmosphere both into the stock sol and the titration cell during a titration forms an important source of error. The apparatus for stocking the sol in an atmosphere of hydrogen gas has been modified by using mercury seal stop-cocks to the exclusion of all rubber connections. The new arrangement (Fig. 1) was found to maintain a vacuum of 750 mm. of mercury for days. The titration cell for simultaneous potentiometric and conductometric measurements [Mukherjee, *et al.*, 1932] has also been modified by the introduction of ground-in mercury seal stirrers (Fig. 2) in order to secure a rapid and efficient intermixture between the sol and the titrant liquid. In view of the high resistances of the systems precautions had to be taken against electrical leakages. The working table and wall plugs were suitably guarded with insulated, earthed metallic shields (copper, except for the table which was covered with galvanised sheets) and similar arrangements were used for the cells and the electrical heating assembly used for the thermostat. All exposed terminals and connecting wires were also similarly protected. These modifications in the experimental arrangement gave e.m.f.'s constant to within 0·1 millivolt and also worked satisfactorily in other regards. A Leeds and Northrup K-type potentiometer and a galvanometer with a current sensitivity of 10^{-11} ampere were used. All measurements were carried out in an electrically heated water thermostat maintained at a temperature 35°C. ±0·05.

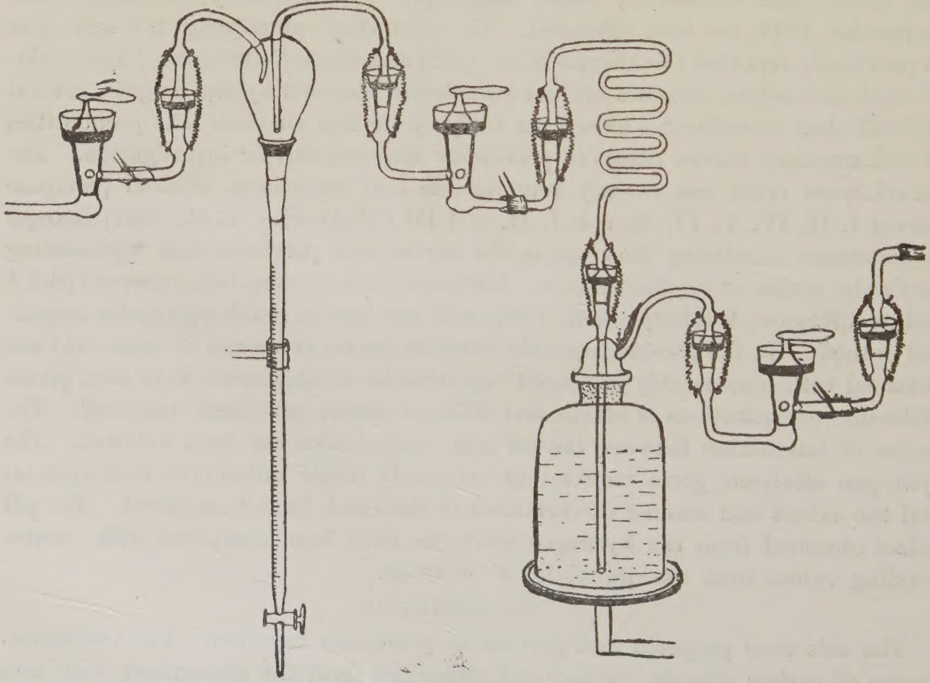


Fig. 1. Stocking arrangement.

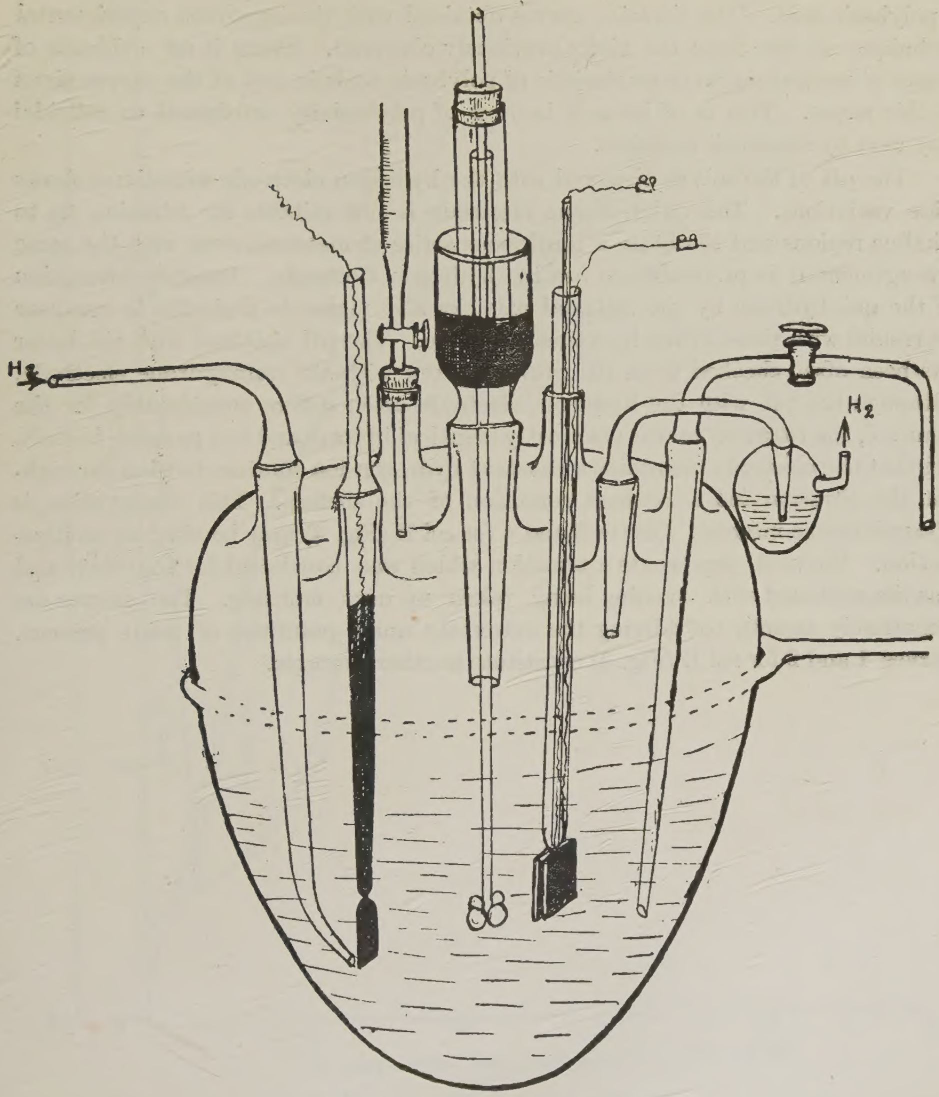


Fig. 2. Titration cell.

REPRODUCIBILITY OF RESULTS

The slopes of the titration curves afford useful information and the reproducibility of the curves is of particular importance. As already stated the curves given in previous papers were often not exactly reproducible and they sometimes showed more than one change in curvature resembling the steps in the curves of a polybasic acid. The titration curves obtained with the improved experimental technique are free from the kinks previously observed. There is no evidence of stages of neutralisation characteristic of polybasic acids in any of the curves given in this paper. This is of interest in view of polybasicity attributed to colloidal clay acid by some soil chemists.

The pH of the sols as observed with the hydrogen electrode sometimes shows wide variations. The quinhydrone electrode is not suitable for titration up to alkaline regions and to obtain a continuous series of measurements with the same arrangement it is preferable to use the hydrogen electrode. Possible adsorption of the quinhydrone by the colloidal particles also makes it desirable to compare its results with those of the hydrogen electrode. The pH obtained with the latter has been often checked as in the previous work with the quinhydrone electrode. Although the pH with the hydrogen electrode often differs considerably for the same sol, the titration curves are mostly identical in form and run parallel, indicating that the electrode maintains a constant hydrogen-ion solution tension throughout the titration and a constant condition of the surface. This observation is of considerable interest. Curves 3 and 4 for sol E (Fig. 4) may be cited as an illustration. Curve 4 represents a titration which was continued for four days and was discontinued each evening being taken up next morning. The curves are remarkably smooth considering the extremely small quantities of acids present. Curves 1 and 2 for sol D (Fig. 3) constitute another example.

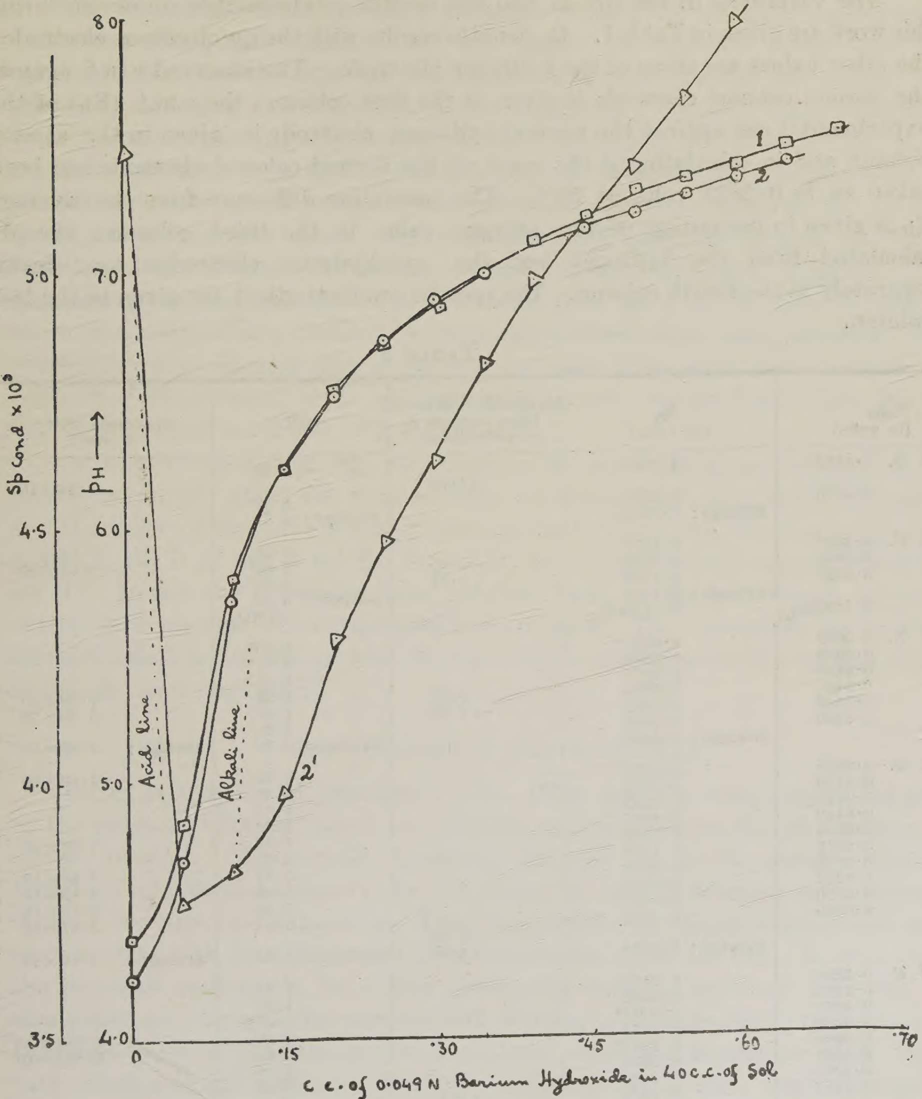


Fig. 3. Silicic acid sol D.

The variations in the pH as also the specific conductivities observed during this work are given in Table I. Q_h denotes results with the quinhydrone electrode ; the other values are those of the hydrogen electrode. The observed e.m.f. against the normal calomel electrode is given in the first column ; the e.m.f. (E_h) of the experimental cell against the normal hydrogen electrode is given in the second column and in calculating it the e.m.f. of the normal calomel electrode has been taken to be 0·2823 volts at 35°C. The maximum difference from the average E_h is given in percentage of the average value in the third column ; the pH calculated from the hydrogen and the quinhydrone electrodes are shown separately in the fourth column. The specific conductivities† are given in the last column.

TABLE I

E_{obs} (in volts)	E_h (in volts)	Maximum difference from average E_h (in percentage)	pH	Sp. conductivity in mhos.
Sol D. 0·5416	0·2593	±1·78	4·24	$5{\cdot}24 \times 10^{-5}$
0·5510	0·2687		4·39	
	average : 0·2640		average : 4·32	
Sol E. 0·4993	0·2170		3·55	
0·5042	0·2219	—0·92	3·63	$3{\cdot}76 \times 10^{-4}$
0·5028	0·2205	+1·23	3·60	
	average : 0·2197		average : 3·59	
0·1895(Q_h)	0·2198(Q_h)		3·59(Q_h)	
Sol F. 0·5089	0·2266*		3·70*	
0·5089	0·2266*		3·70*	
0·5480	0·2657		4·35	
0·5447	0·2624	—0·87	4·29	$5{\cdot}20 \times 10^{-5}$
0·5445	0·2622	+1·18	4·29	$5{\cdot}43 \times 10^{-5}$
0·5426	0·2603		4·26	
	average : 0·2626		average : 4·29	average : $5{\cdot}32 \times 10^{-5}$
Sol G. 0·6095	0·3272		5·36	$1{\cdot}40 \times 10^{-5}$
0·6120	0·3296		5·39	
0·6128	0·3305		5·41	
0·6440	0·3617		5·92	
0·6300	0·3477		5·69	$1{\cdot}39 \times 10^{-5}$
0·6215	0·3392		5·55	
0·6383	0·3560		5·82	$1{\cdot}48 \times 10^{-5}$
0·6248	0·3425		5·60	$1{\cdot}45 \times 10^{-5}$
0·6170	0·3347		5·48	$1{\cdot}45 \times 10^{-5}$
0·6445	0·3622		5·92	$1{\cdot}57 \times 10^{-5}$
	average : 0·3431	—5·56 +4·63	average : 5·61	average : $1{\cdot}46 \times 10^{-5}$
Sol H 0·5230	0·2401		3·94	
0·5275	0·2452		4·01	
0·4920	0·2097*		3·43*	
0·5266	0·2443		4·00	$0{\cdot}891 \times 10^{-5}$
0·5080	0·2257*		3·69	$0{\cdot}891 \times 10^{-5}$
0·5331	0·2508		4·10	
0·5265	0·2442	—1·0	3·99	
0·5297	0·2474	+2·1	4·05	
	average : 0·2455		average : 4·02	
0·1585(Q_h)	0·2508(Q_h)		4·1 (Q_h)	
Sol H/2. 0·5370	0·2547		4·17	$0{\cdot}46 \times 10^{-5}$
0·1466(Q_h)	0·2627(Q_h)		4·30(Q_h)	
Sol H/5. 0·1307(Q_h)	0·2786(Q_h)		4·57(Q_h)	$0{\cdot}21 \times 10^{-5}$
0·1276(Q_h)	0·2817(Q_h)	—0·90	4·61(Q_h)	
0·1264(Q_h)	0·2829(Q_h)	+0·70	4·63(Q_h)	
	average : 0·2811		average : 4·60	
Sol H/10. 0·5352	0·2529		4·14	$0{\cdot}11 \times 10^{-5}$
0·1240(Q_h)	0·2853(Q_h)		4·67(Q_h)	
Sol H/20. 0·5927	0·3104		5·08	$0{\cdot}056 \times 10^{-5}$
0·1099(Q_h)	0·2994(Q_h)		4·90(Q_h)	

* These values have been omitted in calculating the average values.

† The variations in the specific conductivities arise from diffusion from the salt bridge.

The limitations of the hydrogen electrode for activity measurements of such dilute unbuffered acid solutions are well known. It has been found that the agreement between the different measurements is often, though not always, improved by cleansing and re-platinising the electrodes. Where a large number of measurements agree between themselves and the average so obtained also gives a pH in agreement with that of the quinhydrone electrode the absolute value of the pH is reliable to the extent of the variations. In other cases we do not lay stress on the absolute value. The unknown small potential drop at the liquid junction of the saturated potassium chloride bridge introduces a minor error. The activity of the hydrogen ions calculated from the e.m.f. is liable to greater error on account of the functional relationship between these two quantities. The extremely small concentration of the acid has also to be taken into account in estimating the strength of the acid indicated by such comparison. The discussion in the sequel is subject to these remarks.

As already mentioned the variations in the intial pH of the titration curves do not materially affect the total acidities calculated from them as the curvature and the course of the curve is mostly independent of them (see, for example, curves 1 and 2, sol D ; 3 and 4, sol E ; 10 and 11, sol F ; particularly curves 18 and 19, sol H). In fact the calculated total acidities have mostly been found to agree within ± 5 per cent of average values in all cases. The variations are greatest for sol G which is also the sol with the highest pH, the lowest total acid, and which is also the least conducting.

PECULIARITIES OF SILICIC ACID SOLS

It has been observed [Mukherjee *et al.*, 1934] that the total acid taking part in the reaction with the alkali is indefinite and depends on the peculiarities of surface reaction. Theoretically speaking this need not be the common characteristic of all colloidal acids, or, for the matter of that of different colloidal solutions of the same acid substance. Thus the particles of a finely dispersed sol may react rapidly with the alkali when its concentration is not too low. In such cases the processes responsible for a slow interaction with the hydroxyl ions may be eliminated and the colloidal particles will simulate acid molecules in true solution. Different types of titration curves have been observed [Mukherjee *et al.*, 1934] with dilute sols and with sols which are concentrated or have large aggregated particles. When such concentrated sols are diluted the titration curves conform more and more to the form of moderately strong acids. This change in form may, in part, be ascribed to the same reasons as obtain when a weak acid in true solution is sufficiently diluted and its degree of dissociation becomes large. But indications were also obtained that a slower neutralisation of the hydroxyl ions resembling more an adsorption process than the neutralisation of hydrogen ions also takes place. A comparison of the curves for sol A and its dilutions [Mukherjee *et al.*, 1934] may be cited as an illustration. The conductometric titration curves show that in the acid region the slope decreases as the neutral point is approached (sols A/6 and A/11). The conductometric titration curve for sol A/2 indicates adsorption near about the neutral point. At higher alkali concentrations another type of slower neutralisation of hydroxyl ions is conceivable and arises out of the dissolution of the colloidal particles.

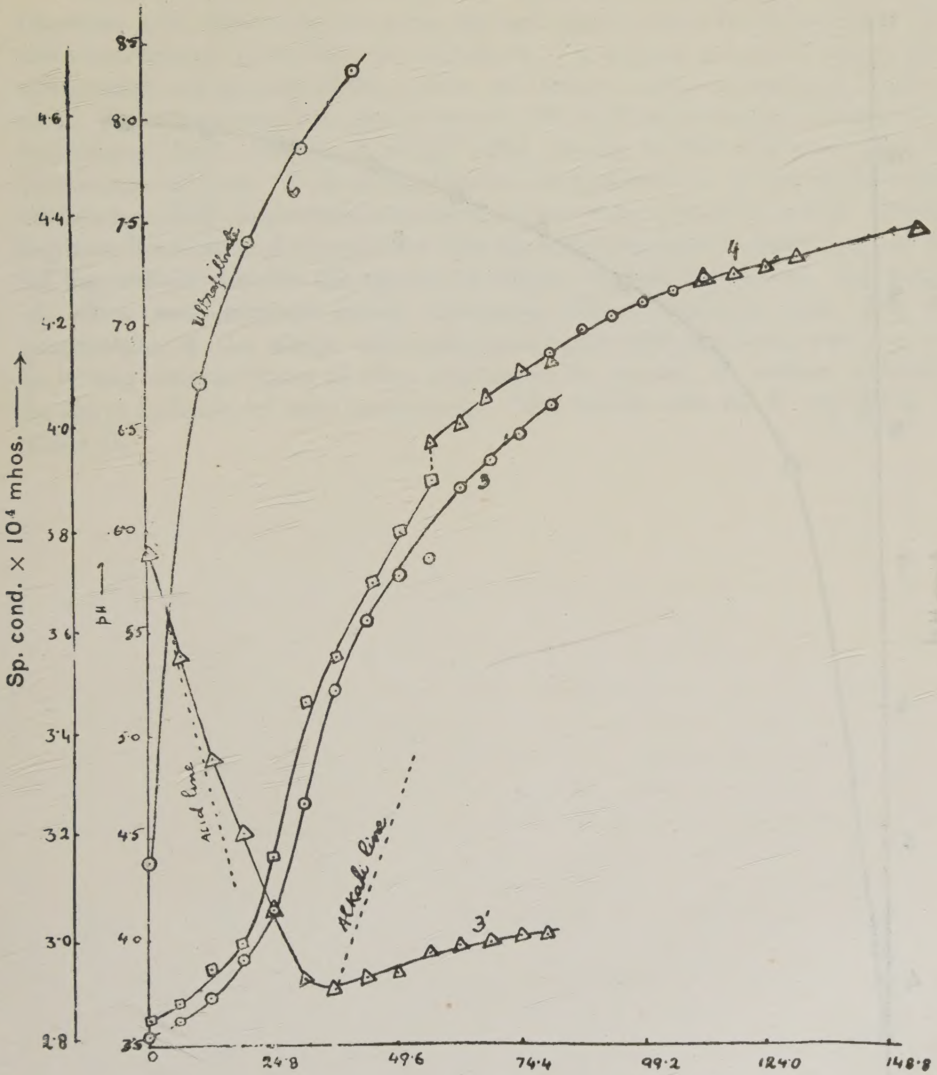
Some of the sols have been found to be more dissociated (Table VI) than others, judging from a comparison of their free and total acidities. The amounts of sodium and hydrogen chlorides contained in them may have some relation to this difference apart from the concentration of the particles. The liberation of acids by salts of alkali and alkaline earth metals of weak and strong acids when they interact with colloidal acids in the sol or gel form is well known and is of general interest, but these interactions do not seem to have been systematically studied.

Sol D

Experiments with this sol were intended to check the efficiency of the experimental arrangement in the way of obtaining reproducible measurements. The potentiometric titration curves 1 and 2, of this sol are remarkably smooth and are almost identical. These curves as also the conductometric titration curve 2′ indicate an acid of a fairly strong character.

Sol E

This sol contained finely-dispersed particles. No aggregates were visible with the naked eye. The ultrafiltrate was obtained from collodion membranes which were previously washed with water of specific conductivity $1 \cdot 34 \times 10^{-6}$ mhos till the wash liquid had a constant specific conductivity of $3 \cdot 0 \times 10^{-6}$ mhos. A nickel plated ultrafilter of Zsigmondy's pattern was used. Traces of nickel were later found to contaminate the ultrafiltrate and the ultrafilter was, therefore, replaced by a porcelain one of the same pattern. ‘ Cella ’ membranes of various pore sizes have been used in later work.



Final conc. of the added alkali (in normality) × 10^5.

Fig. 4. Silicic acid sol E.

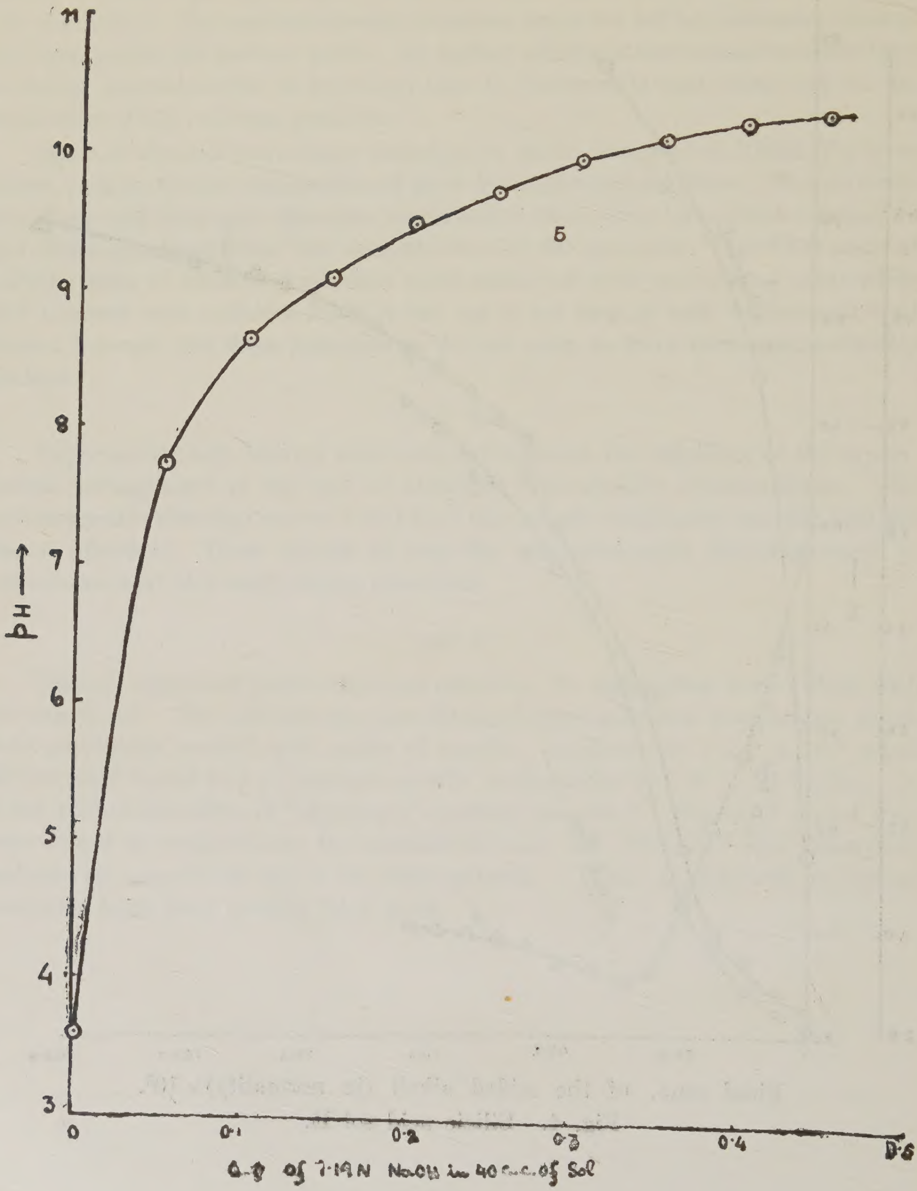


Fig. 5. Silicic acid sol E.

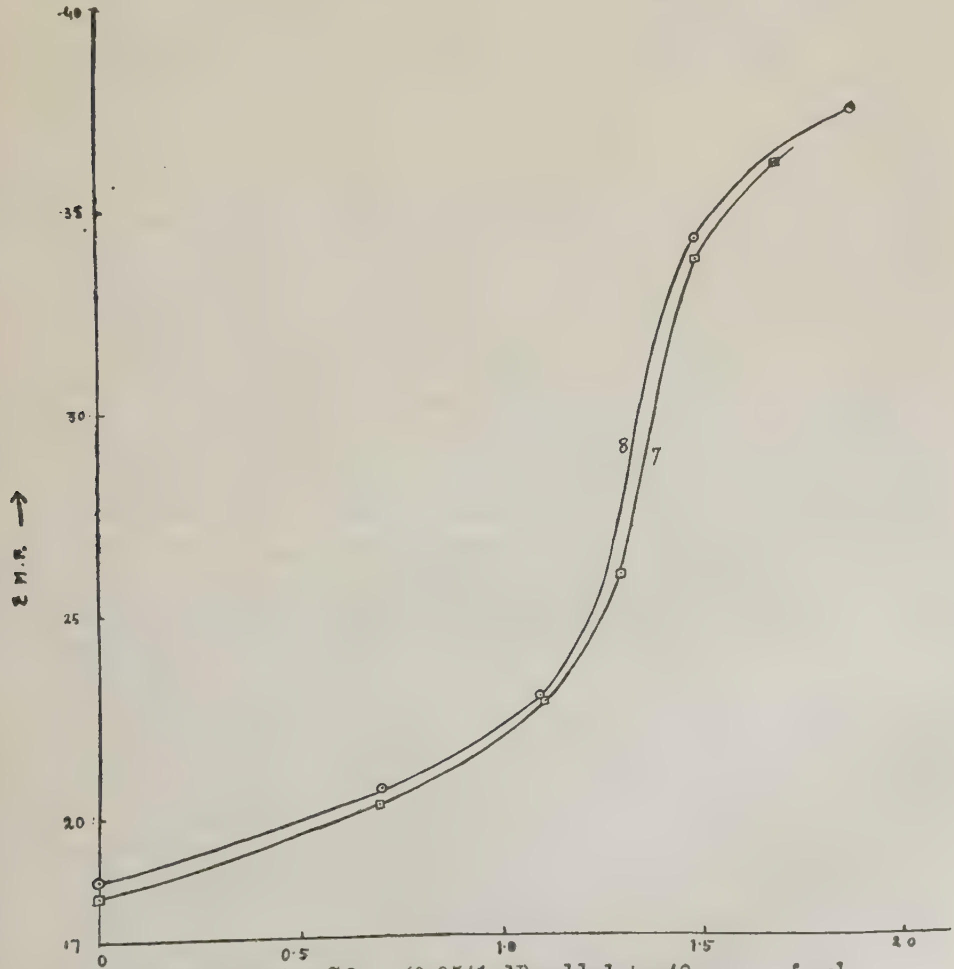
The total chlorine was obtained by both conductometric and electrometric titrations with silver sulphate using Ag-AgCl electrodes in the latter case. The silica and sodium were estimated as follows: A definite volume of the sol after evaporation and ignition of the residue was treated with concentrated sulphuric acid. The residue was then gently heated with additions of drops of concentrated hydrofluoric acid; the loss in weight (after drying) by this treatment gave the percentage of silica. A blank experiment was performed to ascertain the weight of the non-volatile impurities in the same volume of the hydrofluoric acid. Assuming from the method of preparation that the other non-volatile ingredient of the sol was sodium chloride the amount of sodium was calculated from the weight of silica and sulphate before treatment with hydrofluoric acid. For the preparation of the silicic acid sols Merck's pure sodium silicate was used and as it may contain traces of other substances the amount of sodium estimated as above is liable to some uncertainty. The results with sol E are given in Table II.

TABLE II

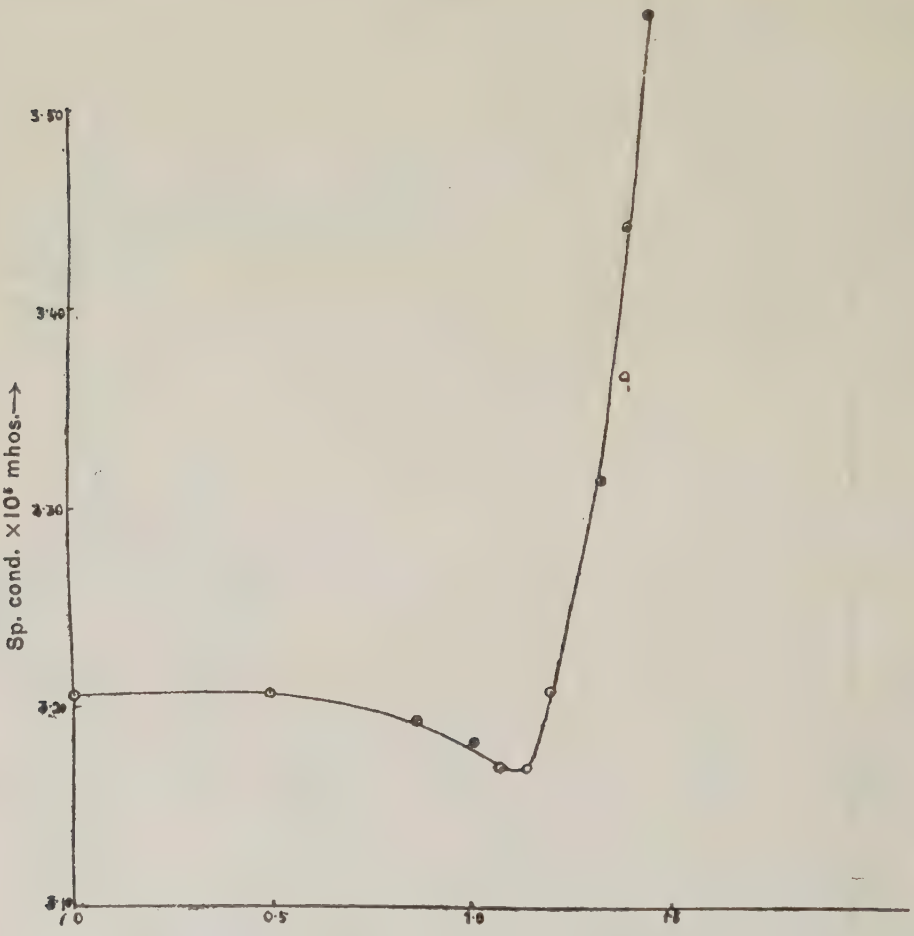
	Gm.-mols. of SiO_2 per litre	Total sodium	Total chlorine	Sp. cond. in mhos × 10^5 (Obs.)	Sp. cond. in mhos × 10^5 (Calc.)	Total acid × 10^5	H-ion conc. × 10^5 (Free acid)	Free acid / Total acid
Ultrafiltrate of sol E	...		0·00153*N* (curve 9)	2·53			4·17	
Sol E . . .	0·116	0·0014*N**	0·00151*N* (curve 7)					
	0·116	0·00146*N*	0·00151*N* (curve 8)	3·76	3·44	30·4 (curve 3)		
	0·115	0·00146*N*				30·0 (curve 4)		
	0·115	0·00146*N*	0·00155*N* (conducto-metric)				25·7 (from average pH Table 1)	0·85
	average : 0·1155	average : 0·00146*N*	average : 0·00152*N*			average : 30·2		

*Rejected.

Curves 3, 3′ and 4 indicate a moderately strong acid character in agreement with the high ratio of free to total acids. The acidity of the ultrafiltrate (*cf.* curve 6) which may also be taken to measure the concentration of the free hydrochloric acid in the sol is about fifteen per cent of the total acid of the sol. The concentration of chlorine in the sol (curves 7, 8, Fig. 6) agrees well with that in the ultrafiltrate (curve 9, Fig. 7). All the chloride is, therefore, present in the intermicellar liquid and completely passes into the ultrafiltrate. The chlorine is contained mostly as sodium chloride and the remainder as free hydrochloric acid. The conductometric titration curve (curve 9) of the ultrafiltrate with silver sulphate has the usual form of the titration of a chloride by a soluble silver salt. The decrease in the conductivity (about 1·2 per cent) towards the end point is partly due to the dilution of the titrant liquid.



c. c. of Ag $_2$SO$_4$. (0·0541 *N*) added to 40 c. c. of sol.
Fig. 6. Sol E.



c. c. of Ag_2SO_4. (0·0541 *N*) added to 40 c. c.
Fig. 7. Ultrafiltrate of Sol E.

The calculated specific conductivity given in column 5 of Table II was obtained as follows : Assuming the sodium and the hydrogen ions to be the only carriers of positive electricity the excess of the sum of their observed concentrations over the concentration of the chlorine ions gives the ' equivalent concentration of the negatively charged silicic acid particles. The observed specific conductivity should be given by the sum of the contributions to the total conductivity due to the individual ion constituents. From the known total concentration of chlorine ions in the sol the concentration calculated per c. c. multiplied by the equivalent conductivity of chlorine ions at infinite dilution at 35°C. gives the contribution of these ions to the specific conductivity. The contributions due to the sodium and the hydrogen ions are similarly obtained, the activity of the hydrogen ions being calculated from the average pH. An equivalent conductivity of 20 has been assumed for the silicic acid particles. The following limiting equivalent conductivities at 35°C. have been assumed for the other ions : $U_{H^{\circ}} = 404$; $U_{Na^{\circ}} = 64$; $V_{Cl} = 93$. It will be found that the observed specific conductivity of the sol is nearly ten per cent higher than the calculated specific conductivity.

The analysis of the ultrafiltrate shows that the strong acid character of the titration curve cannot be attributed to the hydrochloric acid present in the sol. This resemblance with strong acid disappears however when the titration is carried with higher alkali concentrations and up to alkaline regions. Curve 5 (Fig. 5) is typical of a weak acid. The final rise in pH has not been attained though the alkali added would neutralise an amount of acid forty times the total acid given in Table II.* The particles begin to dissolve and this is responsible for the strong buffer action. The adsorption of the alkali or the dissolution of layers of the particle would both explain the peculiarities of many of the conductometric titration curves in the alkali region. They also explain how local changes in concentration simulating steps of dissociation of weak acids may be produced by the disappearance of the alkali by such reactions unless the stirring is vigorous enough to produce a more uniform reaction. The conductometric titration curve 3′ with barium hydroxide on closer examination shows a departure from the slopes of that of a strong acid. For the sake of comparison the theoretical curves for the neutralisation of a strong acid (hydrochloric acid) by the same alkali is given in dotted lines.

The conductometric titration curve resembles, to a certain extent, the neutralisation of a mixture of a strong and a weak acid, the strong acid in this case being hydrochloric acid. The concentration of the strong acid component of the mixture can be calculated from the point of intersection of the salt line of this acid and the initial straight portion of the curve produced. The concentration thus calculated is, however, very much larger than the total acid of the ultrafiltrate (curve 6) which contains all the free hydrochloric acid.

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*More detailed investigations in this direction are in progress.

An initial straight portion followed by a rounding of the curve near about the minimum point is characteristic of the conductometric titration curve of a moderately strong acid. The resemblance disappears however when the titration is carried to alkaline regions, the sharp rise following a round portion being absent. In fact the difference in the slope of the titration curve of the sol after the minimum point has been passed from that of the theoretical curve is even more pronounced in the alkaline than in the acid region. It is interesting to find a similar flattening of the potentiometric titration curve after the sharp rise corresponding with the minimum point of the conductometric titration curve has been passed. A surface reaction in which the alkali is used up is indicated by both sets of curves.

Sol E, on the whole, behaves as a strong acid though not as strong as hydrochloric acid of comparable concentration. The strong acid character is revealed by titration with dilute alkalis only while the titration curve with a strong alkali indicates a weak acid character. The high ratio of free to total acid indicates a strong acid character.

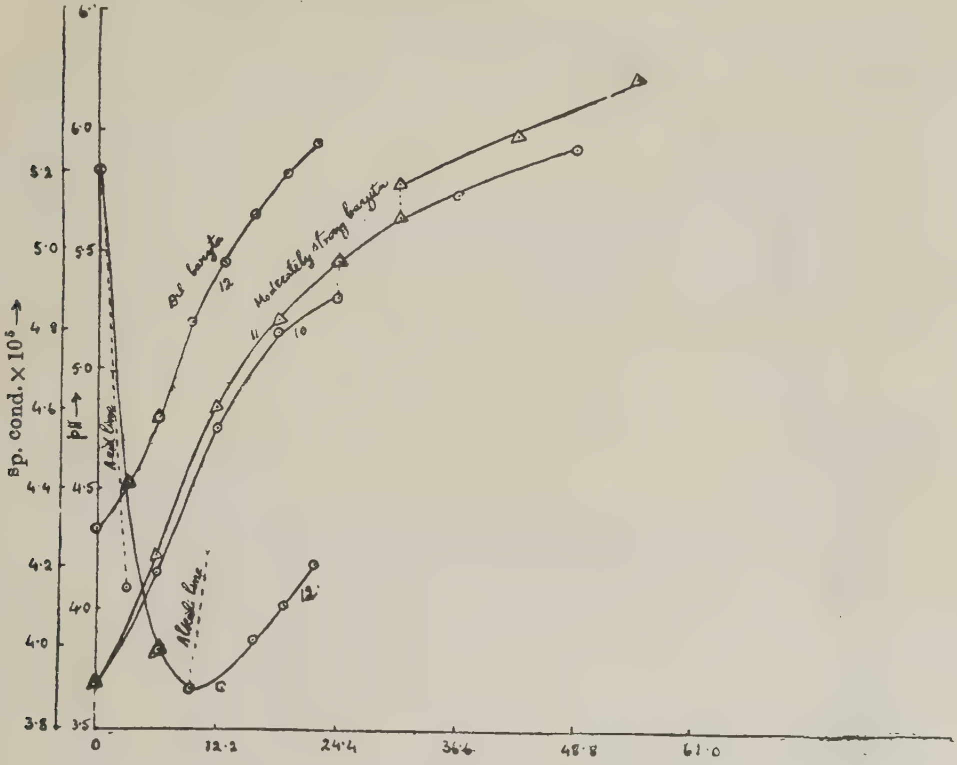
Sol F

Sol F with lower specific conductivity and hydrogen-ion concentration was next used. The results are given below in Table III.

TABLE III

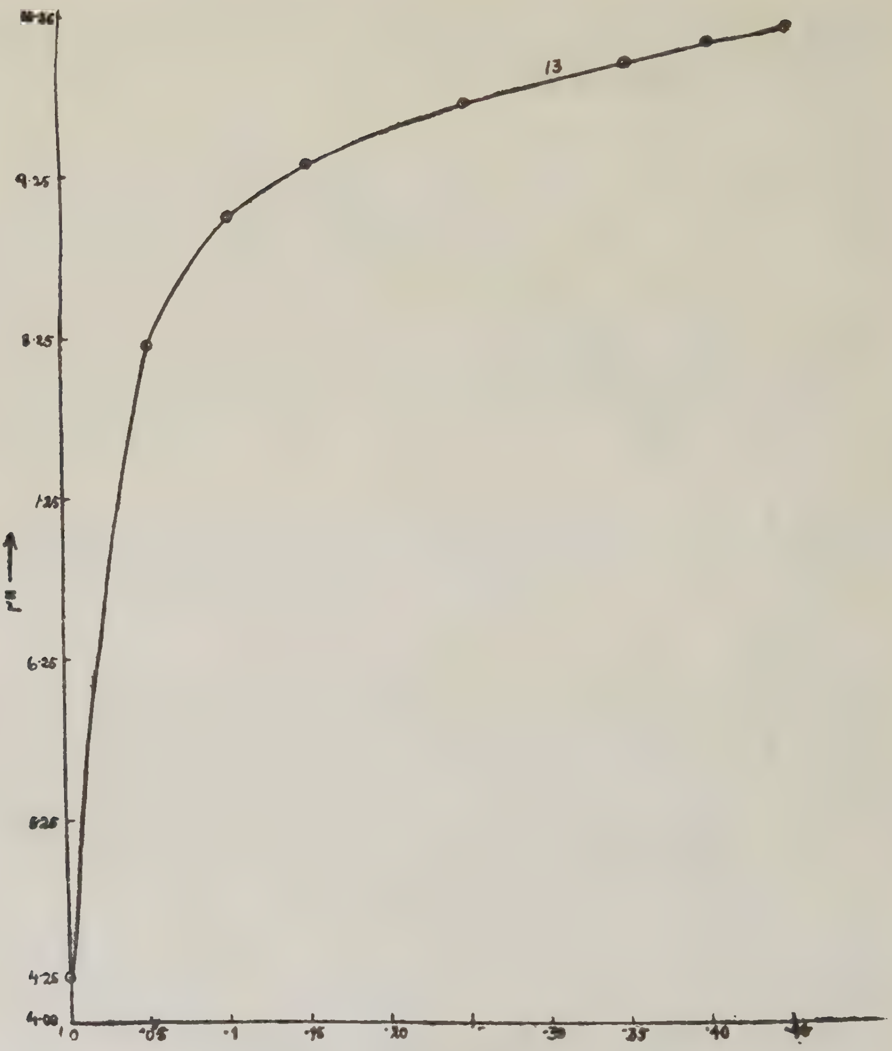
SiO_2 in grm.-mol. per litre	Total sodium in normality ×10^4	Total chlorine in normality ×10^4	Total acid in normality ×10^5	Free acid in normality ×10^5
0·1440	1·10	1·00	7·8 (curves 12 and 12′)	8·1
0·1440	1·05	0·97		

The potentiometric curve 12 (Fig. 8) with dilute baryta resembles that of an acid of medium strength. The conductometric titration curve 12′ gives evidence of buffer action (compare sol E) near about the minimum point. Though not so suitable for calculation of the total acidity for reasons already explained, it gives roughly the same value as curve 12. With an alkali of moderate strength the strong acid character becomes less pronounced (curves 10 and 11) and with more concentrated alkali the curve (curve 13, Fig. 9) becomes typical of a weak acid (compare sol E). The small quantity of the acid involved in the titration and the dissolution of the particles with more concentrated alkali is responsible for the 'weak acid' type of the curve. The initial portions showing a strong acid character would only be observed with dilute alkali. The pH 4·29 from the average e.m.f. of the four measurements which agree between themselves (excluding the two values 3·69 given in Table I) indicates a low dissociation, but if these values are included in calculating the average, a pH 4·09 is obtained in agreement with the total acidity. As no measurement was made with the quinhydrone electrode no reliance can be placed on this agreement.



Equivalents of alkali in 40 c.c. of sol × 10^6.

Fig. 8. Sol F.



c. c. of NaOH (1·19N) added to 40 c. c. of sol.

Fig. 9. Sol F.

The titration of the sol was continued over three days. In agreement with previous observations the initial pH observed next day is always slightly larger than the final equilibrium pH of the previous day.

Sol G

The reproducibility was not satisfactory for sol F. Sol G is more dilute than sol F. Its specific conductivity was about $1 \cdot 5 \times 10^{-5}$ mhos (Table I) and the total acid about 1×10^{-5} grm. ions of H^+ per litre (*vide* curves 14, 15 and 16, Fig. 10). The reliability of the measurements for such sols has an additional interest in relation to the work on suspensions of similar pH by some soil chemists. The curves with dilute sodium, barium and calcium hydroxides are similar and indicate an acid of medium strength. No definite breaks occur. The slopes of the curves indicate (see later) that adsorption or surface reaction plays a proportionately larger part than the neutralisation of hydroxyl ions by hydrogen ions. The free acid ($2 \cdot 4 \times 10^{-6}$ grm. ions of H^+ per litre, calculated from the average pH given in Table I) appears to be considerably smaller than the total acid but the comparison loses its significance for the above reason and the wide variation in the pH values. The titration curve of this sol with strong alkali resembles those of sols E and F and shows a weak acid character. The experimental difficulties are still more pronounced in agreement with the very low concentration of the acid. The results on the whole are of interest as they show that besides a neutralisation process an adsorption or dissolution process should be considered.

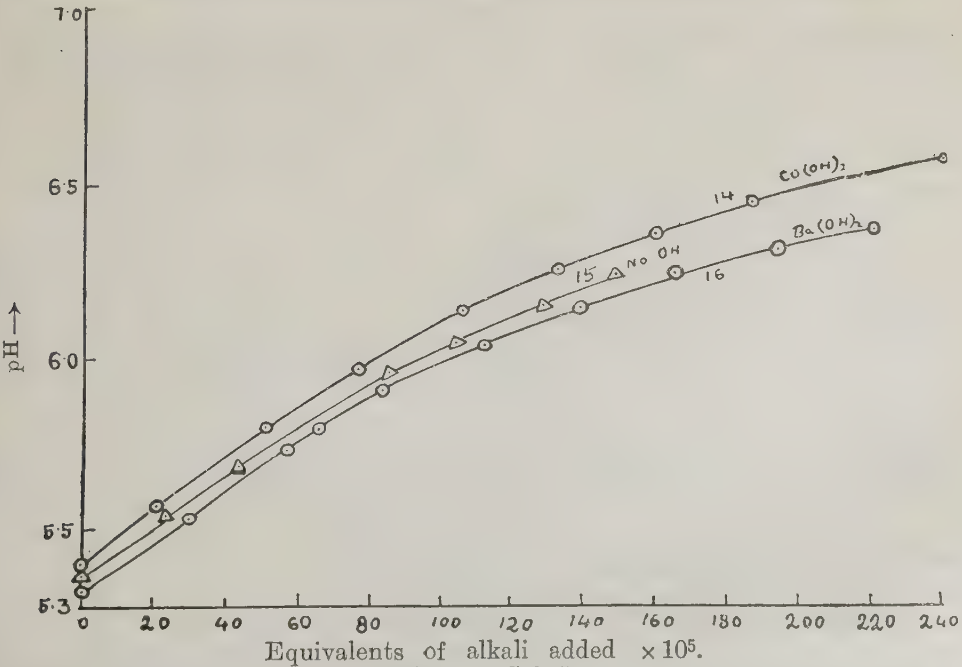


Fig. 10. Sol G.

Sol H and its dilutions

The preceding results show that sols having a pH below five give more reliable data. Sol H comes within this range. The results are given in Table IV.

TABLE IV

	Conc. of SiO_2 in grm-mol. per litre	Total acid $\times 10^5$ in normality	pH	H ion conc. $\times 10^5$ (free acid) in normality	$\frac{\text{Free acid}}{\text{Total acid}}$	Sp. cond. in mhos $\times 10^5$	Total chlorine $\times 10^5$
Sol *H* . .	0·233 0·233	14·4 (curves 18 & 18′ with NaOH) 14·4 (curve 19 with NaOH) 15·5 (curves 20 & 20′ with $Ba(OH)_2$) 16·9 (curve 22 with dil. NaOH) average : 15· 3	4·02 (Pt_{H_2}) 4·10 (Q_H) average 4·06	8·7	0·57	8·91	5·0
Ultrafiltrate of sol H		2·24 (curves 23 & 23′)				2·51	5·00

TABLE IV (*a*)

Dilutions of sol H

	Total acid $\times 10^5$ in normality	pH	H ion conc. $\times 10^5$ (free acid) in normality	$\frac{\text{Free acid}}{\text{Total acid}}$	Sp. cond. in mhos $\times 10^5$	Total acid $\times$ dilution
Sol H/2 . . .	7·2 (curve 25 ; Fig. 14)	4·16 (Pt_{H_2}) 4·30 (Q_H) average : 4·23	5·8	0·80	4·6	14·4
Sol H/5	3·1 (curves 26 and 26′ ; Fig. 15)	4·56, 4·61, 4·63 (Q_H) average : 4·60 4·09 (Pt_{H_2})	2·5	0·81	2·1	15·5
Sol H/10	1·6 (curves, 27 and 27′ ; Fig. 16)	4·67 (Q_H) 4·14 (Pt_{H_2})	2·0		1·1	16·0
Sol H/20	0·8 (curves 28 and 28′ ; Fig. 17)	4·91 (Q_H) 5·09 (Pt_{H_2}) average: 5·0	1·0	1·00	0·56	16·0

The total acid of the ultrafiltrate (curve 23Fig ;11) is again about sixteen per cent of that of the sol. It appears that the method of preparation of the sol, which involves a neutralisation of the sodium silicate by the same proportion of hydrochloric acid and dialysis till no trace of chlorine is found in the dialysate with silver nitrate, leaves almost the same proportion of hydrochloric acid in the sol. The chlorine-ion concentration of the ultrafiltrate is again the same as that of the sol, *i.e.*, the whole of the chlorine comes out in the ultrafiltrate.

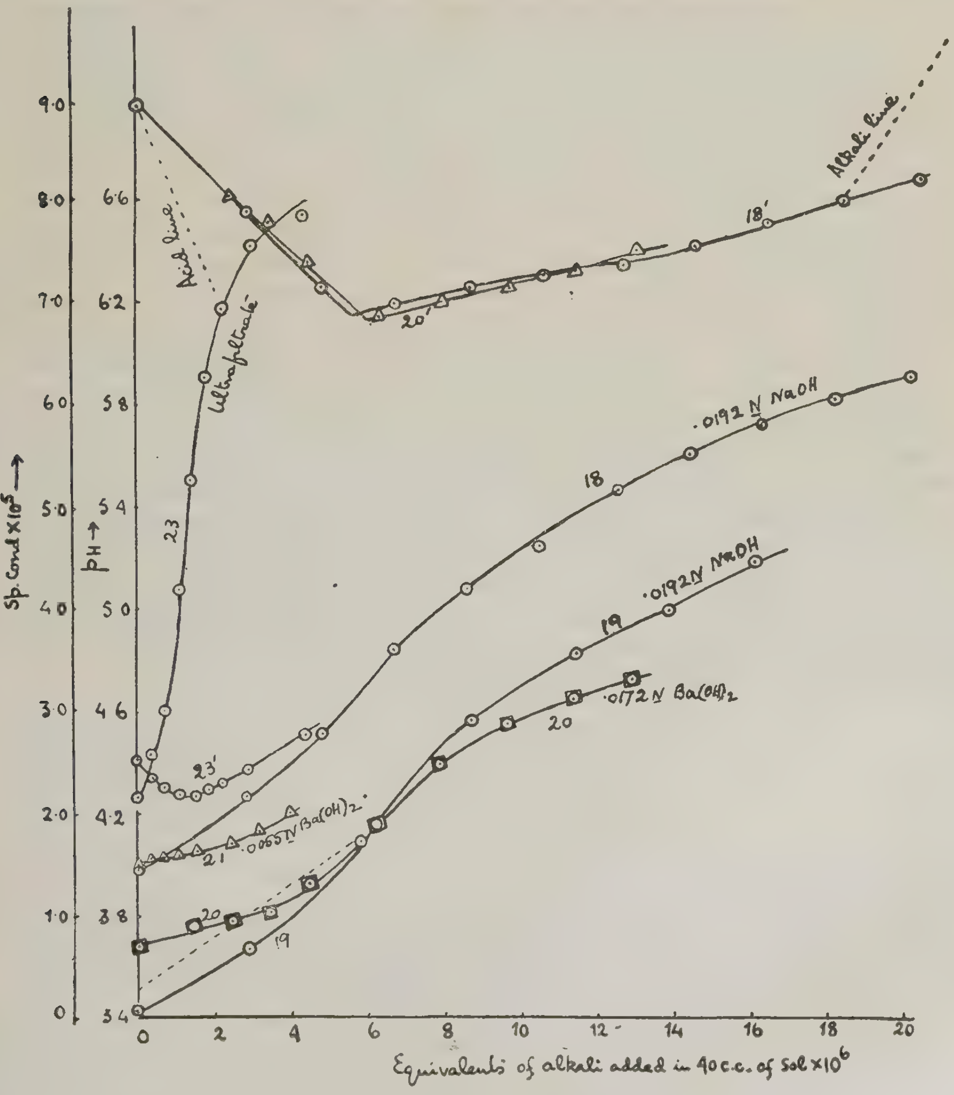
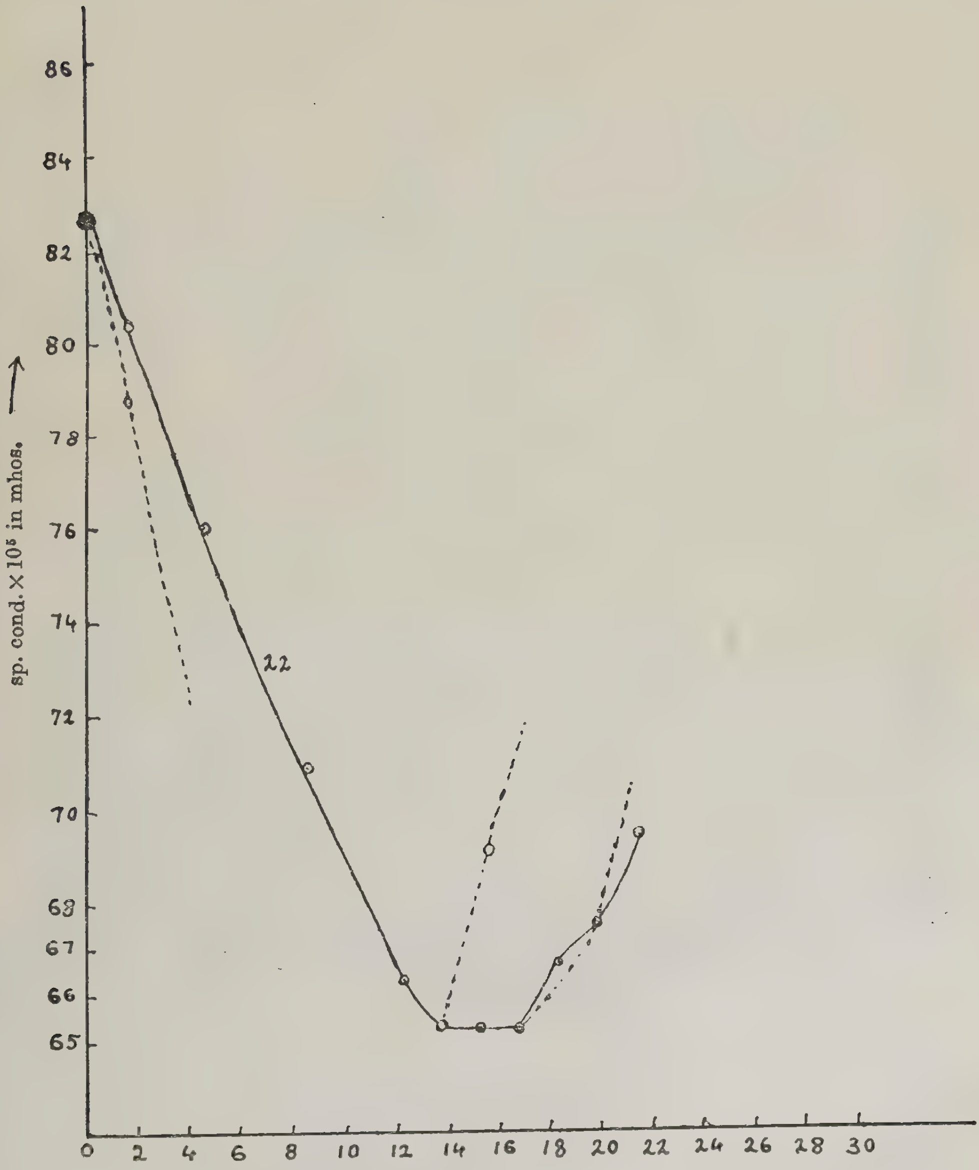


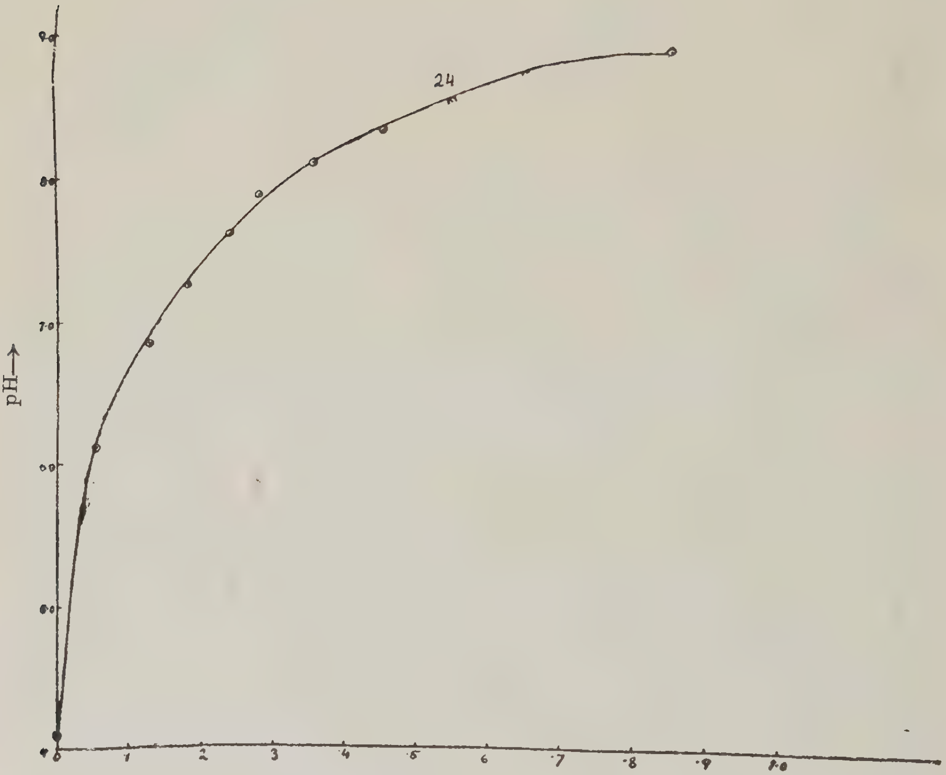
Fig. 11. Silicic acid sol H.

From Fig. 11 it appears that the curves are fairly reproducible. The two curves for sodium hydroxide (curves 18 and 19) are parallel for reasons discussed earlier. The conductometric curves for both sodium and barium hydroxides show a change in curvature a little after the turning point has been passed. A reaction with the alkali is clearly indicated (compare sol E). The potentiometric curve with barium hydroxide is different from that with sodium hydroxide. The former reacts more strongly than the latter, the differences being particularly pronounced in the initial stages where a strong buffer action is shown with barium hydroxide. It is interesting to find, however, that the total acidities with the two alkalis are not much different. The total acid with very dilute caustic soda (curve 22 ; Fig. 12) is higher than with moderately strong caustic soda. The initial convexity characteristic of strong acids is obtained only with dilute alkali (curves 18 and 20). The titration curve 24 (Fig. 13) with strong alkali is similar to that of sol E and indicates a weak acid character. The particles dissolved in the region corresponding to the flat portion of the curve. Conductometric titration curve 22 for sol H was obtained with a Vreeland oscillator as the source of alternating current (1000 cycles) and a Leeds Northrup set of Curtis Coil resistances and bridge. The curve is very smooth and the horizontal portion of the curve is of interest. The alkali is very dilute. The conductometric curves resemble those observed with many other sols [Compare sols A/2, A/6, A/11, Mukherjee *et al.*, 1934]. In considering the curvature the observed slope should be compared with the theoretical slopes (dotted lines) for neutralisation of hydrochloric acid under similar conditions. The departure from the theoretical slope is, as with sol E, more pronounced in the alkaline than in the acid region. At high alkali concentrations a dissolution of the particles requires to be considered. When the total acid concentration is large, the convexity in the acid region is less pronounced, possibly because the neutralisation process forms the major proportion of the reaction with the alkali. The slopes, compared with the theoretical slopes, show more definitely the adsorption process near and after the neutralisation point.



Equivalents of alkali ($0{\cdot}00695N$ NaOH) in 40 c. c. of sol × 10^7

Fig. 12. Sol H.

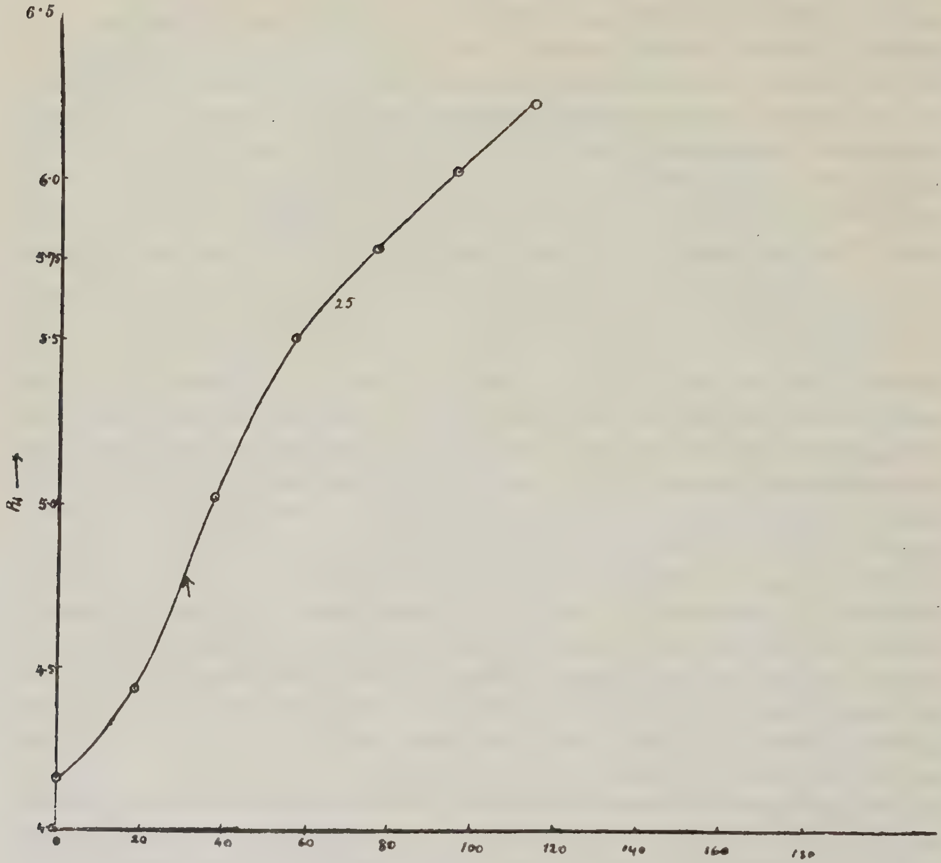


c. c. of strong NaOH (0·34N) in 40 c. c. of sol.

Fig. 13. Sol H.

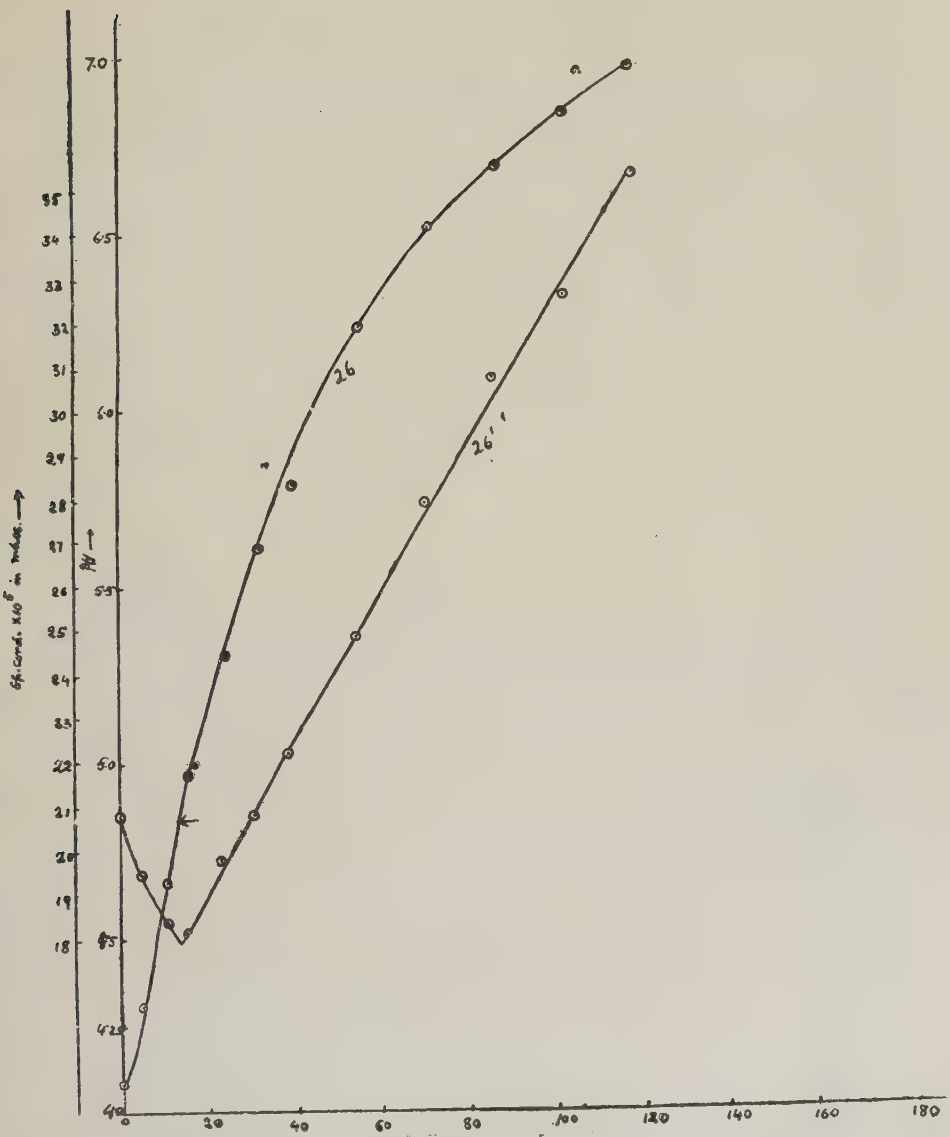
The total acid decreases almost proportionately with the dilution up to sol H/5. With sols H/10 and H/20 the total acid calculated from the curve is liable to larger percentage errors in view of the extremely small quantities of the acid present. The same alkali was employed in the titration of the different dilutions of sol H and the manner of addition was also nearly the same as previous experience with silicic acid sols [Mukherjee *et al.*, 1934] indicates that the total acid depends to some extent on these factors. Thus the total acid for sol H is larger with dilute alkali than with more concentrated alkali. The change in pH due to the same quantity of the alkali being added, $\frac{\delta \mathrm{pH}}{\delta c}$, is comparatively large with the more dilute sols H/10 and H/20 and the location of the exact point of inflexion between the two points between which $\frac{\delta \mathrm{pH}}{\delta c}$ is maximum is more difficult. The total acid changes regularly on dilution as is shown by the agreement between the ‘ average ’ total acidity of sol H and the total acid of the diluted sols multiplied by their dilution. This regularity does not preclude the existence of an adsorption process. The amount of alkali adsorbed perhaps varies proportionately with the dilution, depending as it should on the particle concentration and the equilibrium concentration of the alkali.

The specific conductivity changes very regularly with the dilution and this illustrates the satisfactory nature of the arrangements. The free acidities for sols H and H/2 are fairly reliable in view of the good agreement between the average values from the hydrogen electrode and the value from the quinhydrone electrode. The free acidities of sols H/5, H/10, and H/20 obtained from the quinhydrone electrode have been taken to be more reliable and have been used for calculating the degree of dissociation, but the absolute values cannot be relied upon to the same extent as for sols H and H/2. The sol H is about sixty per cent dissociated. The strength of the acid character would be better understood by plotting log r against log c where r is the ratio of free to total acid of the different dilutions of the sol and c their respective total acid concentrations. A similar plot for hydrochloric acid solutions of comparable concentrations would facilitate the comparison.



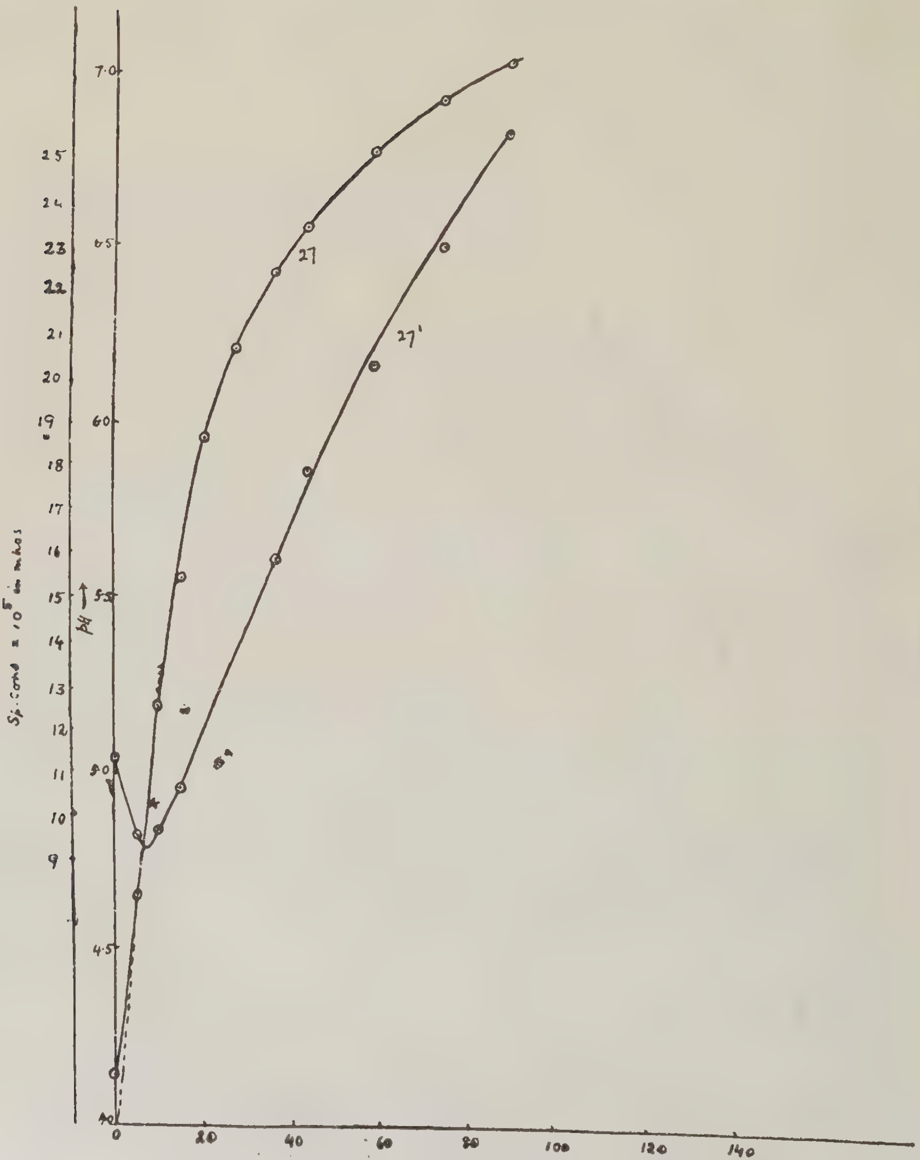
Equivalents of alkali × 10^7 in 40 c. c. of sol.

Fig. 14. Silicic acid sol H/2.



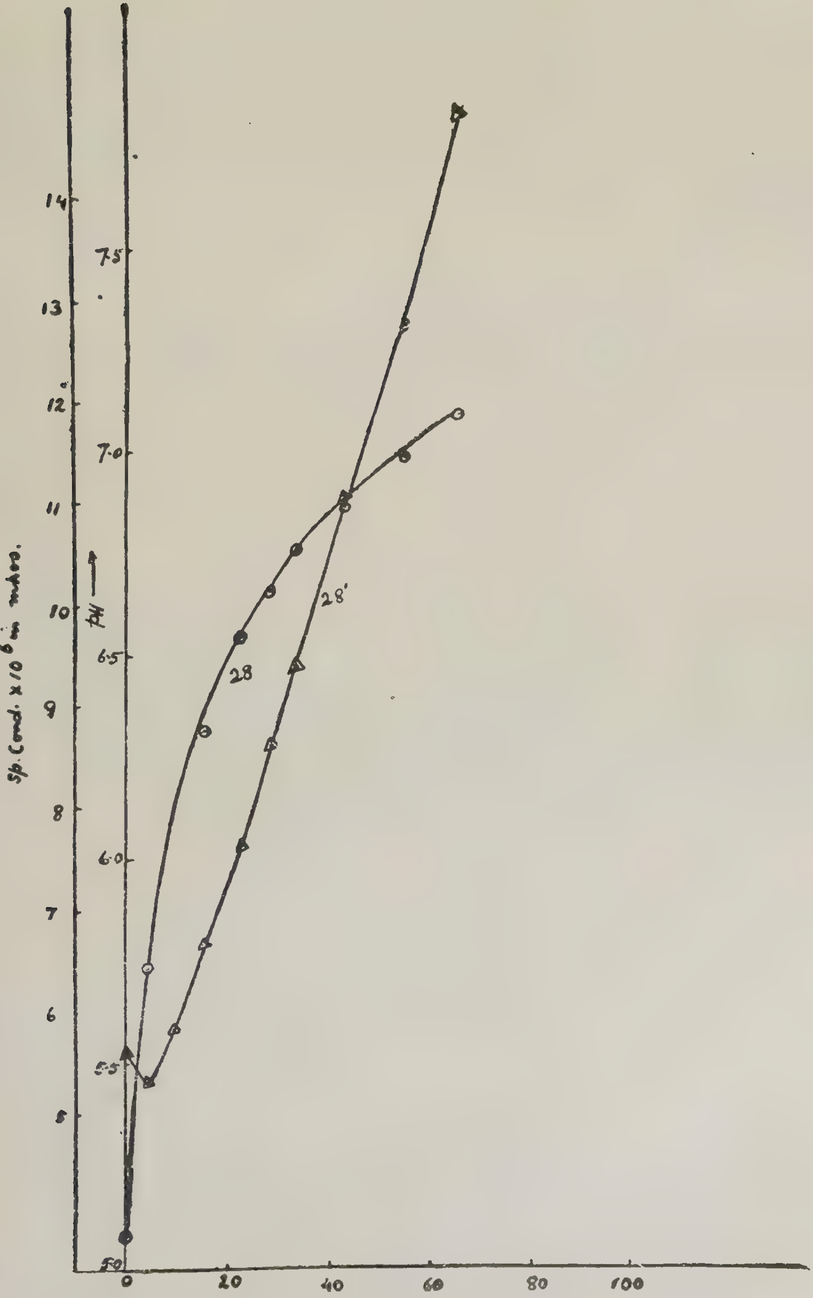
Equivalents of alkali (NaOH) × 10^7 in 40 c. c. of sol.

Fig. 15. Silicic acid sol H/5.



Equivalents of alkali (NaOH) × 10^7 in 40 c. c. of sol.

Fig. 16. Silicic acid sol H/10.



Equivalents of alkali (NaOH) × 10^7 in 40 c. c. sol.
Fig. 17. Silicic acid sol H/20.

Silicic acid sol I

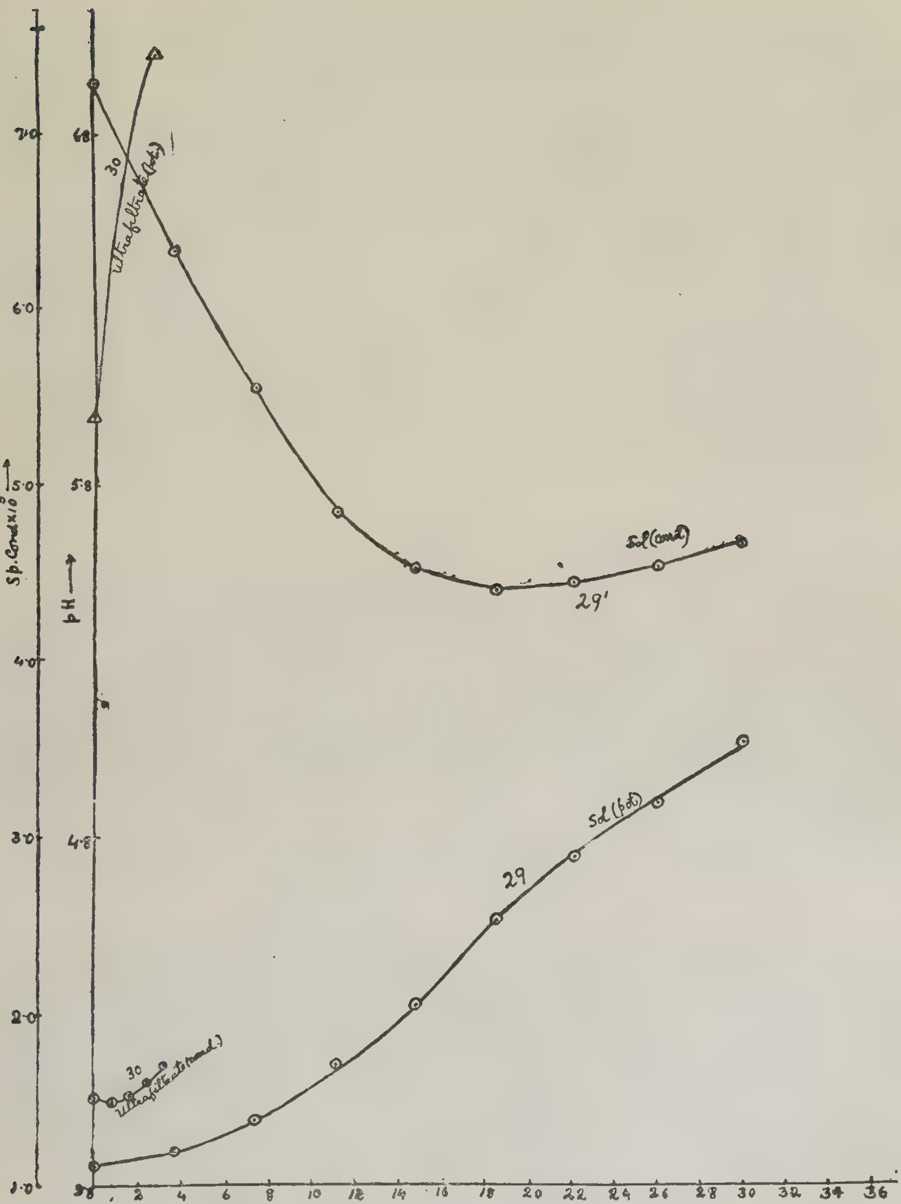
Dissolved hydrochloric acid contributes an appreciable fraction (15 per cent) to the observed total acids of sols E and H. It was thought that prolonged dialysis might remove these last traces of hydrochloric acid from the sol. Accordingly sol I was dialysed for a much longer interval of time against repeated changes of conductivity water towards the end of the operation. The results with this sol. are given below :

Sol I

TABLE V

	Grm—mols. of SiO_2 per litre	Total chlorine	Sp. cond. in mhos $\times 10^5$	pH	H-ion conc.	Total acid
Sol I	0·235	Negligible	7·3	3·85	$1{\cdot}41\times10^{-4}N$	$16{\cdot}6\times10^{-5}N$ (curves 29 and 29′; Fig. 18)
Ultrafiltrate of sol I .	...	Negligible	1·52	6·2	$6{\cdot}31\times10^{-7}N$	$0{\cdot}8\times10^{-5}N$ (conductometric curve 30′ ; Fig. 18)

The analysis of the ultrafiltrate shows that dissolved acids contribute a very negligible fraction (curves 30 and 30′ ; Fig. 18) to the observed total acid. The chlorine-ion concentration is nil and the ultrafiltrate is practically neutral. The titration curve 29 (Fig. 18) with sodium hydroxide indicates a moderately strong acid character of the sol ; the conductometric titration curve 29′ is similar to that of sol H. Sol I thus presents the same broad features as sol H.



Equilibrium concentration of alkali × 10^5.

Fig. 18. Silicic acid sol I.

In a recent paper Rabinowitch and Kargin [1935] have expressed the view that the acid character attributed by previous workers to silicic acid sols should really be referred to the presence of dissolved acids in the sol. In support of their view they quote some experimental results with silicic acid sols purified by prolonged electrodialysis which show that these sols showed practically neutral reaction and had conductivities comparable with that of pure water. As pointed out by Valko [1935] the above behaviour of Rabinowitch's sols was due to their extremely low silica contents. Both silicic acid sols H and I are about sixty times more concentrated than Rabinowitch's sols. As already pointed out the small total acid of the ultrafiltrate of sol I in particular, shows that the strong acid character of this sol cannot be due to free acids present in the sol. Also unpublished work from this laboratory with sufficiently concentrated electrodialysed silicic acid sols shows that these sols have the same order of magnitude of total acidities as sols H and I and they present the same broad features.

BASICITY OF COLLOIDAL ACIDS

Colloidal acids have been considered to be polybasic acids in true solution [Pauli and Valko: Elektrochemie der kolloide, 1929]. The titration curves given in this paper bear no evidence of a polybasic character. They are rather typical of monobasic acids. A closer inspection, however, will show that this resemblance is only superficial. The slope of the titration curve of a weak monobasic acid at the point of half neutralisation is independent of the dissociation constant of the acid and is given under certain conditions of titration by Auerbach and Smolczyk [1924].

$$\frac{\delta \text{pH}}{\delta x} = \frac{1{\cdot}74}{xa}$$

where xa denotes the volume of the alkali required to reach the equivalence point. The theoretical slope calculated from the equation (dotted lines in Fig. 12 ; sol H) seldom agrees with the slope. The discrepancy is more pronounced with the titration curve with barium hydroxide. This shows that the silicic acid sols cannot be treated as weak monobasic acids in true solution. Adsorption by and dissolution of the particles are, on the other hand, sufficient to account for the forms of the titration curves.

"STRENGTH" OF SILICIC ACID SOLS

In Table VI the free and total acids of different silicic acid sols where the value of the free acid could be fairly relied upon have been summarised and "dissociation constants" calculated from them given in the last two columns.

TABLE VI

	Total acid	Free acid	$\frac{\text{Free acid}}{\text{Total acid}}$	K_1	K_2
Sol I (curve I, part I, of this series).	$0{\cdot}0034N$	$0{\cdot}00234N$	$0{\cdot}688$	$5{\cdot}5\times10^{-3}$ $7{\cdot}0\times10^{-3}$ $13{\cdot}2\times10^{-3}$	$5{\cdot}16\times10^{-3}$
Sol A/2 (curve III, part II of this series) . .	$0{\cdot}0065N$	$0{\cdot}0059N$	$0{\cdot}91$	$3{\cdot}56\times10^{-1}$ $3{\cdot}87\times10^{-1}$	$5{\cdot}89\times10^{-2}$
Sol A/11 (curve VII, part II, this series) . .	$0{\cdot}00087N$	$0{\cdot}00084N$	$0{\cdot}97$	$6{\cdot}11\times10^{-2}$	$2{\cdot}3\times10^{-3}$
Sol D (curves 1 and 2 . .	$0{\cdot}00009N$	$0{\cdot}000048N$	$0{\cdot}53$	..	$5{\cdot}4\times10^{-3}$
Sol E (curve 3) .	$0{\cdot}00030N$	$0{\cdot}000257N$	$0{\cdot}85$	$3{\cdot}9\times10^{-3}$ $5{\cdot}9\times10^{-3}$	$1{\cdot}75\times10^{-3}$
Sol H . . .	$0{\cdot}000153N$	$0{\cdot}000087N$	$0{\cdot}57$	$4{\cdot}6\times10^{-5}$ $1{\cdot}29\times10^{-4}$	$1{\cdot}15\times10^{-4}$
Sol H/2 (curve 25).	$0{\cdot}000072N$	$0{\cdot}000058N$	$0{\cdot}80$	$6{\cdot}4\times10^{-5}$	$1{\cdot}9\times10^{-4}$
Sol H/5 (curve 26)	$0{\cdot}000031N$	$0{\cdot}000025N$	$0{\cdot}81$	$2{\cdot}56\times10^{-5}$ $3{\cdot}24\times10^{-4}$	$1{\cdot}1\times10^{-4}$

Inspite of the strong acid character of the titration curves colloidal silicic acid is often dissociated to the extent of about sixty per cent though the total acid concentration is of the order of 10^{-5} grm. ions of hydrogen per litre. It appears also that the free and total acids do not depend solely on the concentration of the silica.

The 'dissociation constants' K_1 calculated from the following equation have been given in column 5 of Table VI.

$$\mathrm{pH} = \mathrm{p}K_1 + \log \frac{[\mathrm{Salt}] + [A']}{[\mathrm{Acid}] - [A']}$$

The pH corresponding to a given addition of the alkali is obtained from points in the neighbourhood of half neutralisation ; the concentration of the colloidal salt, [salt], is taken to be equivalent to the alkali added ; the concentration of the unneutralised acid, [acid], is given by the difference of the total acid as obtained from the titration curve and the concentration of the added alkali ; $[A']$ denotes the concentration of the anions dissociated from the unneutralised acid and is roughly given by the product of the concentration of the unneutralised acid with the ratio of free to total acids ; the concentration of unneutralised undissociated acid is given by [acid] $-[A']$. In view of the high ratio of free to total acids the more simple Henderson equation appears to be inapplicable for calculating the constants. In the last column of table 'dissociation constants', K_2 calculated from the equation $K_2 = \frac{\alpha^2 . c}{1 - \alpha}$

have been given. α is given by the ratio of free to total acids and the molecular concentration c by the total acid as obtained from the titration curves, the acid being assumed to be monobasic.

Though conveying an approximate idea of the ' strength ' of silicic acid sols the calculated ' dissociation constants ' are not constants in any strict sense of the term. In a previous paper [Mukherjee *et al.*, 1934] it has been observed that the 'dissociation constants' of colloidal acids have a fictitious significance because of the indefiniteness, among other things, of the total acid taking part in the reaction. Thus the total acid has been found to depend on the nature and concentration of the alkali (compare the curves with dilute and concentrated alkalis given in this paper), the time allowed for interaction [Mukherjee *et al.*, 1932] and on other factors.

For an interpretation of the titration curve of an acid in relation to the strength of its acid character one need consider, besides the dissociation constant, the solubility of the salt formed and its degree of hydrolysis. The hydrolysis of a salt is expected to be greater with a weak alkali than with a stronger one, and on this ground a titration curve with barium hydroxide should indicate a greater rise of pH than a curve with sodium hydroxide. The titration curves of silicic acid sol H show that this is contrary to our observation. Work from this laboratory with palmitic acid sols show that the titration curve with barium hydroxide shows a strong buffer action which is not shown by the curve with sodium hydroxide. In the case of an acid in true solution its neutralisation by an alkali requires different theoretical treatment according as the salt formed is in solution, or, forms an insoluble precipitate. In the neutralisation of a silicic acid sol with barium hydroxide we are concerned with the adsorption of barium ions on the oppositely charged primarily adsorbed ions on the surface of the colloidal particles whose dimensions are large compared to that of ordinary molecules or atoms. The formation of a secondarily adsorbed layer of barium ions on the surface has to be distinguished from the formation of an insoluble precipitate, *e.g.*, barium silicate. The ' ion pairs ' formed by adsorption may not constitute a distinct phase. The solubility of such a ' salt ' does not convey any clear meaning.

The interaction of ions on the surface of colloidal particles is more readily understood from the standpoint of the adsorbabilities of ions as postulated by Mukherjee [1921]. According to this view the particles of colloidal acids owe their negative charge to the presence on the surface of a fixed layer of primarily adsorbed anions. Corresponding to the number of these ions an equivalent number of oppositely charged ions (in this case hydrogen ions) remain in the liquid near the surface. A certain number of these hydrogen ions may remain fixed on the surface by electrostatic or chemical valency forces forming an electrically adsorbed layer while the remaining hydrogen ions form the ' mobile sheet ' of the double layer. On the addition of an alkali besides the simple neutralisation of the 'mobile' hydrogen ions by the hydroxyl ions the cation of the added alkali may displace some of the electrically adsorbed hydrogen ions which can then neutralise further quantities of hydroxyl ions. The hydroxyl ions of the added alkali may be primarily adsorbed by chemical forces with a consequent increase in the negative charge of the particles which can now drag in more cations into the double layer. The greater the adsorption of the cations the more pronounced will be the buffer action. The relative 'electrical' adsorbabilities of the cations is a function of their valencies, mobilities and states of hydration. On this basis alone the adsorbability of barium ions should be stronger than that of sodium ions and would account for the stronger buffer action of silicic acid sols towards barium hyroxide than towards sodium hydroxide (compare sol H).

ACTION OF NEUTRAL SALTS ON COLLOIDAL ACIDS

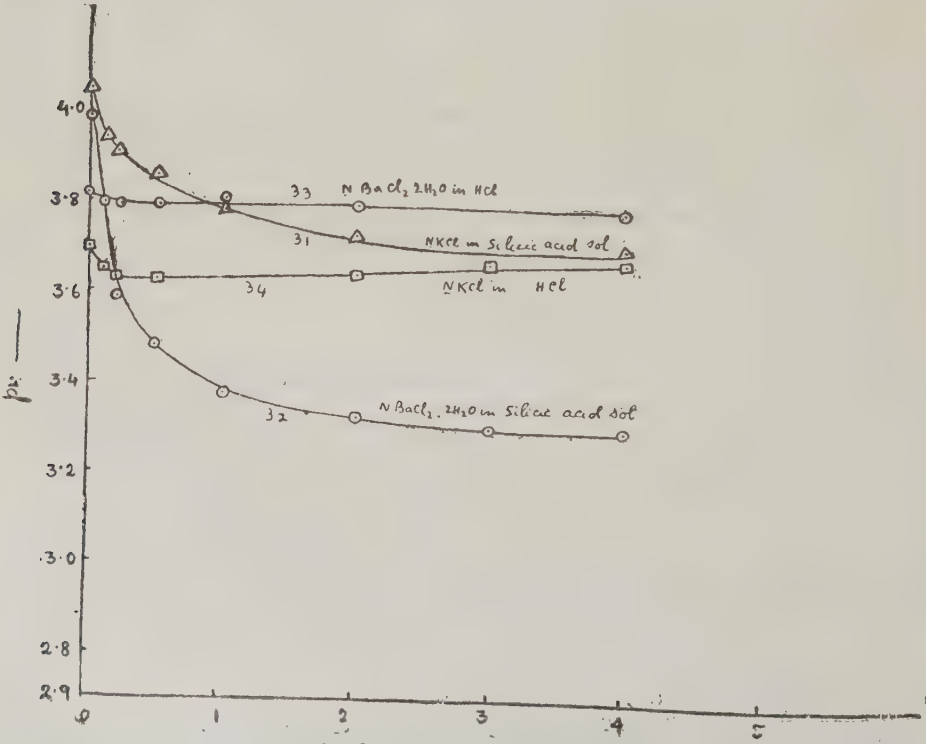
The interaction of soil with neutral salts is of fundamental interest to soil chemistry. A distinction should be made between the sol and the gel state. In the latter case any displaced hydrogen ion whether it comes from the mobile sheet of the double layer or from the primarily or secondarily adsorbed layers will always show an increase in the acidity of the supernatant water. In the case of the sol a free hydrogen ion in the mobile sheet so displaced by a cation of a salt of a strong acid will not alter the pH unless the activity coefficient of the hydrogen ion in the mobile layer is different from what it is in the intermicellar liquid. An increase in the hydrogen ion concentration denotes displacement of hydrogen ions from the primarily or secondarily adsorbed layer. Analyses of the ultrafiltrate and estimation of the total acidity in the presence of the salt would afford valuable information of the interaction.

To sols H and I were added gradually increasing amounts of neutral solution of potassium and barium chlorides and the resulting changes in pH are given below.

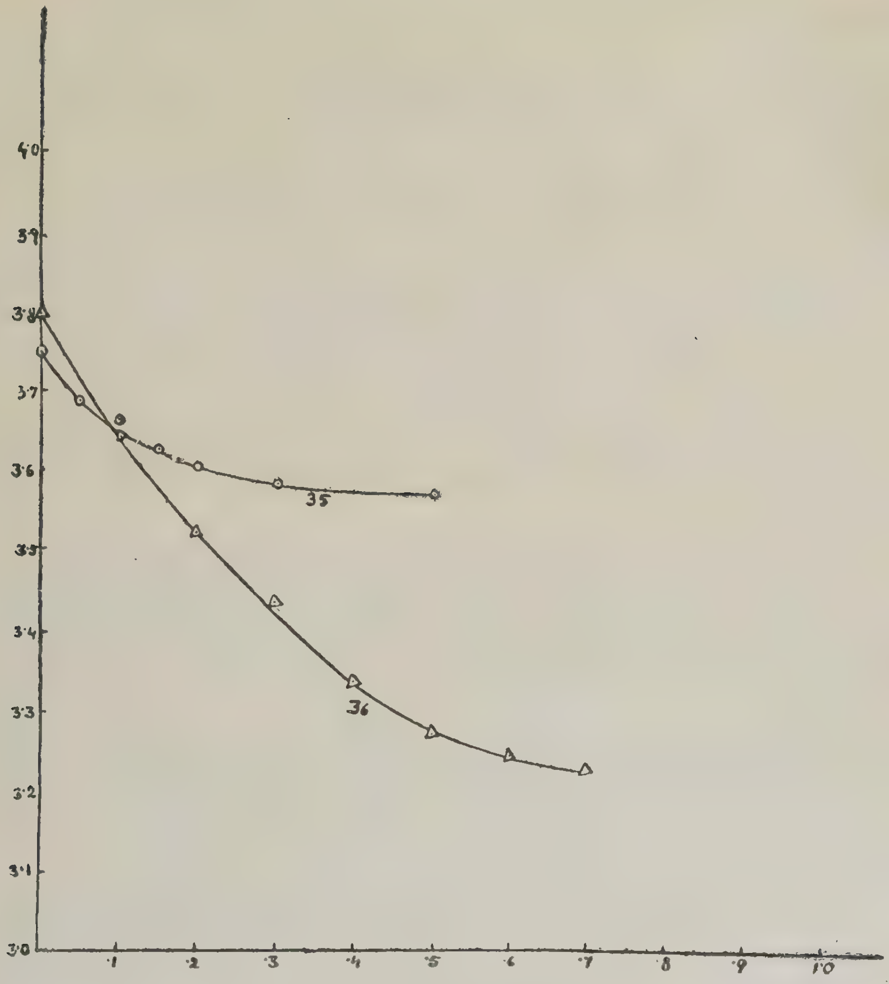
TABLE VI

	Total acid as obtained by titration with alkalis (no neutral salt being added)	Initial pH without addition of salt	pH after addition of salt	Conc. of salt added
Sol H	$15{\cdot}2\times10^{-5}N$	4·0 4·05	3·3 (curve 31) 3·70 (curve 32)	0·09 N $BaCl_2$ 0·09 N KCl
Sol I	$16{\cdot}6\times10^{-5}N$	3·60 3·75	3·20 (curve 35) 3·58 (curve 36)	0·013 N $BaCl_2$ 0·013 N KCl

The curves 31 and 32 (Fig. 19) for sol H and curves 35 and 36 (Fig. 20) for sol I show that barium chloride decreases the pH to a greater extent than potassium chloride. For comparison the pH of a hydrochloric acid solution of approximately the same original pH as the sol when titrated in a similar manner, is given in curves 33 and 34 (Fig. 19). The increase in the hydrogen-ion concentration on the addition of barium chloride is about 300 per cent for sol H and 150 per cent for sol I. In fact more acid appears to come out on the addition of barium chloride than the total acid obtained by titration with alkalis. The results are significant in that they bring out prominently the indefinite character of the total acid and the important part played by the cation in reactions in colloidal acid systems. Analysis of the ultrafiltrate and estimation of the total acid in the presence of the salt would afford valuable information regarding the interaction. The estimation of the total acids of silicic acid sols in the presence of the salt is difficult as the sols rapidly set under these conditions. The following table will show that the hydrogen-ion concentration and the total acid of the ultrafiltrate of sol I—the ultrafiltrate being obtained after addition of barium chloride to the sol—are considerably higher than those of the ultrafiltrate and somewhat grater than that of the pure sol. This indicates that the barium ions, in consequence of their stronger electrical adsorbability, displace some of the electrically adsorbed hydrogen ions into the intermicellar liquid.



c. c. of salt solution added in 40 c. c.
Fig. 19. Sol H.



c. c. of normal salt solution added to 40 c. c. of sol.

Fig. 20. Silicic acid sol I.

	pH	H+ ion conc. in normality	Total acid	Conc. of $BaCl_2$
Ultrafiltrate of sol I without addition of $BaCl_2$ to the sol . . .	6·2	$6 \cdot 31 \times 10^{-7}$	$0 \cdot 8 \times 10^{-5} N$	*nil*
Ultrafiltrate (sol I with $BaCl_2$) . . .	3·98	$1 \cdot 05 \times 10^{-4}$	19×10^{-5}	$0 \cdot 059 N$

A detailed discussion of this aspect will be taken up in a future publication.

SUMMARY

The electrochemical properties of a number of silicic acid sols have been studied with special reference to the nature of their titration curves with solutions of alkalis and neutral salts.

The titration curves with dilute alkalis point to a strong acid character while with more concentrated alkalis a weak acid character is revealed, the particles dissolving in the higher alkaline regions. Analyses of the ultrafiltrates of the sols show that their strong acid character cannot be wholly attributed to dissolved acids present in the sol.

The titration curves give evidence of surface reactions distinct from the simple neutralisation of an acid by a base. Adsorption by and dissolution of the particles can, in most cases, account for the forms of the titration curves.

Neutral salts liberate acids from the sols to an extent depending on the 'electrical adsorbabilities' of the cations.

The authors' thanks are due to the Imperial Council of Agricultural Research for enabling one of them (R. P. M.) to carry out this work as a Research Assistant and for other facilities.

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ON THE NATURE OF REACTIONS RESPONSIBLE FOR SOIL ACIDITY

PART V. TITRATION CURVES OF CLAY ACIDS

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(Received for publication on 18th October 1935)

(With eleven text-figures)

The ' hydrogen clay ' obtained by replacing the exchangeable cations of soil by hydrogen ions possesses acidic properties. Several points of view have been suggested to explain the acid character. On titration with alkalis the clay acids have been found by Bradfield to yield titration curves resembling those of monobasic acids [Bradfield, 1927] while some authors have attributed a polybasic character to these acids from observations of more than one ' break ' in the titration curves. According to Bradfield the curves, though not as steep at the inflection point as those of simpler acids, show well-defined endpoints using both conductometric and potentiometric methods.

Against a true acid character of the clay acids is the fact that their titration curves with different strong bases can scarcely be superimposed. Oakley's observation [Oakley, 1927] that superposition is possible provided that the titrations are carried out in the presence of semi-normal solutions of the chlorides of the corresponding bases is interesting as it constitutes a difference in behaviour of the clay acids from that of acids in true solution. The discrepancy regarding the non-equivalence of different baseshas been referred to complex side reactions*e.g.* hydrolysis arising from the relatively large ratio of soil to the reacting base as obtain in the usual procedure of electrometric titrations of weak acids when the acid is taken in the titration vessel and the alkali added from outside [Bradfield, 1924]. Using the reverse procedure in which the hydrogen clay was added to the base Bradfield obtained the same total acidity with different bases. Bradfield's method as also the one adopted by Hissink [1925] in which the soil is placed in excess baryta and the amount of alkali reacted upon obtained by titrating the excess alkali, have been criticised by Schofield [1931] on the ground that at high pH's in the presence of alkali cations soluble aluminates and silicates are formed while with the alkaline-earth cations the aluminates and silicates being insoluble form a layer over the colloidal clay particles. The instability of the particles in the alkaline region thus makes it difficult to obtain, by the above methods, definite information regarding their acid character. The difficulty in finding the endpoint of titration has led to the suggestion that the process is essentially one of adsorption in which both the cations and the hydroxyl ions are adsorbed. This view seems to be supported by a large amount of experimental evidence among which mention may be made of Pennyquick's observation [1930] that hydroxyl ions disappear by a sort of chemical combination with acid groups on the surface as also of Mattson's experiments [1928] showing that when a 'hydrogen clay' is treated with calcium hydroxide the gain in weight of the oven-dried clay corresponds to an adsorption of calcium hydroxide as a whole and not to a simple replacement of hydrogen ions by calcium ions.

In previous papers of this series [Mukherjee *et al.*, 1931, 1932 and 1934] the electrochemical behaviours of individual colloidal ingredients of the soil —acidic (silicic acid sols) and basic (aluminium hydroxide sols)—, have been discussed in the light of experimental results concerning mainly with their titration curves with acids and alkalis. Work with actual clay acids had been deferred pending these investigations with the simpler systems as it was thought that the results obtained thereof would be helpful towards a better understanding of the nature of the more complicated reactions responsible for soil acidity. This preliminary work with pure systems has now sufficiently advanced to justify investigations with actual clay acids. In the present paper the features presented by a number of clay acids isolated from different soil samples have been discussed with special reference to their titration curves with alkalis and solutions of neutral salts.

EXPERIMENTAL

Method of preparation of the clay acids.—The soil was air-dried, powdered and sieved as usual. The organic matter was destroyed by exhaustive treatment with six per cent hydrogen peroxide after which the material was vigorously shaken for several hours with a large volume of water in a motor-driven shaker. The coarse suspension thus obtained was allowed to settle for two to three days. On centrifuging the supernatant liquid with a Sharple's Supercentrifuge a fine stable suspension was obtained. The corresponding 'hydrogen clay' was prepared either by electrodialysis or by leaching with dilute hydrochloric acid followed by washing with conductivity water till the wash liquid showed no visible turbidity with an acidified solution of silver nitrate. In later work acid clays prepared by the hydrochloric acid treatment were electrodialysed to ensure removal of any free hydrochloric acid from the sol.

The following table gives the source and the method of preparation of the different clay acids.

Sol	Source	Method of preparation
A . .	Silt from Ganges water (sediments at the bottom of storage tanks containing Ganges water).	Suspension obtained bycentrifuging only not followed by any acid treatment or electrodialysis.
A_1 . .	Ditto . .	Acid treatment followed by leaching with water to remove any free hydrochloric acid.
B . .	Dacca (Bengal) virgin soil (collected from a depth of 0″—6″).	Ditto.
B_1 . .	Ditto . .	Acid treatment followed by leaching with water and then electrodialysis.
C . .	Dacca (Bengal) cultivated soil (unmanured crop ; collected from a depth of 0″—6″).	Electrodyalisis only.
C_1 . .	Ditto . .	Acid treatment followed by leaching with water and then electrodialysis.

The sol was in all cases stocked in an atmosphere of hydrogen and the arrangement as described in a previous paper [Mukherjee *et al.*, 1932] was used for this purpose. The titration cell for simultaneous conductometric and potentiometric measurements was also as previously used [Mukherjee *et al.*, 1932] with the modification that ground-in mercury seal stirrers were employed to ensure a rapid and thorough intermixture between the sol and the titrant liquid. A Leeds and Northrup potentiometer reading directly to 0·1 millivolt and a galvanometer with a current sensitivity of 10^{-8} ampere were used for potentiometric measurements with sols A and A_1. With the remaining sols a Leeds and Northrup K-type potentiometer and a galvanometer with a current sensitivity of 10^{-11} ampere were employed. Electrolytic hydrogen purified as already described [Mukherjee *et al.*, 1932] was used. All measurements were carried out in an electrically heated water thermostat maintained at a temperature of 35°C. ± 0·05. In view of the high resistances of the systems special precautions had to be taken to guard against electrical leakages which are particularly troublesome during the rainy season [Mukherjee *et al.*, 1936.]

REPRODUCIBILITY OF RESULTS

In connection with previous work with silicic acid sols it was found that the pH values of the sols sometimes showed marked variations. The clay acids appear to behave in an analogous manner in this respect though the variations are not so wide as the following Table I will show. The pH obtained from the hydrogen electrode has, as in previous work, been often checked with the quinhydrone electrode. Inspite of appreciable initial differences the titration curves of a given sol with the same alkali run almost parallel and are generally identical in form. The initial differences arising, probably from a poisoning of the electrode, do not affect the total acidity values calculated from the curves. The point is illustrated, among others, by curves 3 and 4 for sol A_1, and curves 16 and 17 for sol C.

Table I shows the variations in the pH and the specific conductivities of the different sols. The observed e. m. f. against the normal calomel electrode is given in the first and second columns ; the e. m. f. of the experimental cell against the normal hydrogen electrode is given in the third and fourth columns, the e. m. f. of the normal calomel half element being taken to be 0·2823 volt at 35° C. The maximum difference from the average e. m. f. is given in percentage of the average value in the fifth column ; the sixth and seventh columns give the pH calculated from the hydrogen and quinhydrone electrode separately. The specific conductivities are given in the last column.

TABLE I

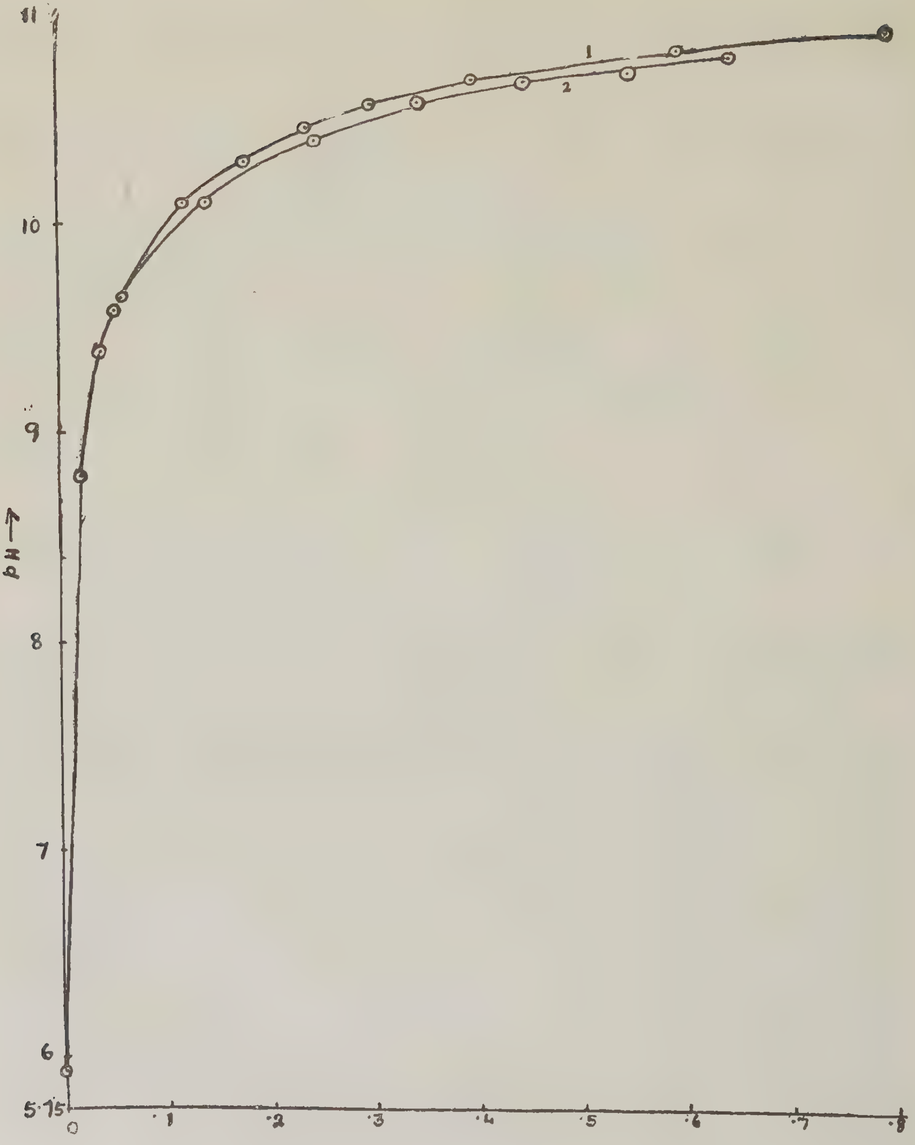
	E_{obs}	E_{obs}	E_h	E_h	Maximum difference from average e. m. f. with hydrogen electrode (in percentage)	pH		
	Hydrogen electrode in volts	Quinhydrone electrode in volts	Hydrogen electrode in volts	Quinhydrone electrode in volts		Hydrogen electrode	Quinhy-drone electrode	Sp. conductivity in mhos
Sol A—								
	0·6450		0·3627			5·93		
	0·6480		0·3657			5·98		
	0·6530		0·3707			6·07		
	0·6550		0·3727			6·10		
			average 0·3680		—1·4 +1·1	average 6·02		
Sol A_1—								
	0·5500		0·2677			4·38		
	0·5530		0·2707			4·43		
	0·5480		0·2657			4·35		
	0·5460		0·2637			4·31		
	0·5450		0·2627			4·30		
			average : 0·2661		—1·3 +1·3	average 4·35		
Sol B—								
	0·6080		0·3257			5·33		
	0·6140		0·3317			5·43		
	0·5900		0·3077			5·03		
		0·0890		0·3203				5·24 × 10^{-5}
			average 0·3235		—4·9 +2·5	average 5·29		
S B_1—								
	0·5850		0·3027			4·95		
	0·5805		0·2982			4·88		
	0·5830		0·3007			4·95		
	0·5902		0·3097			5·04		
	0·5800		0·3077			4·87		
	0·5785		0·2962			4·85		
			average 0·3006		—1·4 +2·4	average 4·92		

TABLE I—*contd.*

E_{obs}	E_{obs}	E_h	E_h		pH		
Hydrogen electrode	Quinhydrone electrode	Hydrogen electrode	Quinhydrone electrode	Maximum difference from average e. m. f. with hydrogen electrode (in percentage)	Hydrogen electrode	Quinhydrone electrode	Sp. conductivity in mhos
Sol C—							
0·4935		0·2112			3·46		
0·5050		0·2227			3·64		
0·5070		0·2247			3·67		$5 \cdot 75 \times 10^{-5}$
0·4955		0·2132			3·49		$5 \cdot 24 \times 10^{-5}$
0·5090		0·2267			3·71		$5 \cdot 42 \times 10^{-5}$
0·4920		0·2097			3·43		
	0·1900		0·2193			3·59	
	0·1895		0·2198			3·60	
		average			average		
		0·2180		—3·9	3·58		
				+4·0			
Sol 1—							
0·5880		0·3057			5·00		
0·5963		0·3142			5·14		$2 \cdot 09 \times 10^{-5}$
0·5975		0·3152			5·16		$2 \cdot 21 \times 10^{-5}$
0·5817		0·2994			4·90		$2 \cdot 15 \times 10^{-5}$
	0·1038		0·3055			5·00	
		average			average		
		0·3086		—0·9	5·05		
				+1·1			

GANGES SILT

Sediments collected from the bottom of storage tanks containing Ganges water were air-dried, powdered, and sieved as usual, and after removal of organic materials with six per cent hydrogen peroxide the colloidal portion was separated with the aid of a Sharple's Supercentrifuge. As it was intended to investigate, in the first instance, the acid properties, if any, of the sediments as they occur in nature neither any preliminary acid treatment nor electrodialysis was taken recourse to in the preparation of the sol (sol A). Curves 1 and 2 (Fig. 1) represent the results of potentiometric titration of this sol with alkalis. The sol shows a practically neutral reaction and the curves are characteristic of very weak acids ; the flat portions of the curves correspond with the region where the sol coagulated. In contrast however, with a true acid character the final ascending portion is absent. The flat portions appear to indicate an adsorption of the alkali by the coagulum in agreement with the observations of previous investigators [Bradfield, 1924] regarding the strong adsorbing power of soil colloids for alkalis in the alkaline region.



c. c. of moderately strong Ba $(OH)_2$ (0·297*N*) added to 25 c. c. of sol.

Fig. 1. Sol A, Ganges silt.

On account of the low acid reaction of the above sol a portion was coagulated with 0·05 *N* hydrochloric acid and the coagulum leached several times with this acid ; the residue was then washed on an ultrafilter with conductivity water till the ultrafiltrate showed no visible turbidity with an acidified solution of silver nitrate. On now vigorously shaking the residue with conductivity water a concentrated suspension was obtained which on centrifuging gave a fine stable suspension (sol A_1).

The titration curves 3 and 4 were obtained with dilute alkali. The results are summarised in the following table :

TABLE II

	Total acid $\times 10^5$ in normality	pH (average)	Free acid in normality	$\frac{\text{Free acid}}{\text{Total acid}}$	Cl-ion concentration $\times 10^5$
Sol A_1 . .	33·2 (curve 3) . 31·1 (curve 4) .	4·35	$4{\cdot}5\times10^{-5}$	·14	5·00
Ultrafiltrate of Sol A_1 . .	. .	5·4	$4{\cdot}0\times10^{-6}$	. .	5·00
Sol $A_1/4$ . .	7·5 (curve 5) .				

The titration curves 3 and 4 (Fig. 2) indicate a strong acid character. The curves are fairly smooth,—the breaks indicating a polybasic character observed by some previous investigators are not in evidence. The chlorine-ion concentration of the sol calculated from the observed e. m. f. of the cell Ag-AgCl/·01 *N* HCl/sat. KNO_3/sol/AgCl-Ag is the same as that of the ultrafiltrate. In view of the weak acid reaction of the ultrafiltrate and the small chlorine-ion concentrations it is not possible to refer the observed acid character of the sol to the presence of free acids in the intermicellar liquid. The strong acid character disappears on titrating with concentrated alkalis (curve 8 ; Fig. 4) ; the particles coagulate and the strong buffer action in the region corresponding with the flat portion of the curve indicates a surface reaction involving the disappearance of considerable amounts of the alkali*. The clay sols, in this respect, appear to behave in an analogous manner with silicic acid sols [Mukherjee *et al.*, 1936] with the difference that with concentrated alkalis the clay particles coagulate instead of dissolving.

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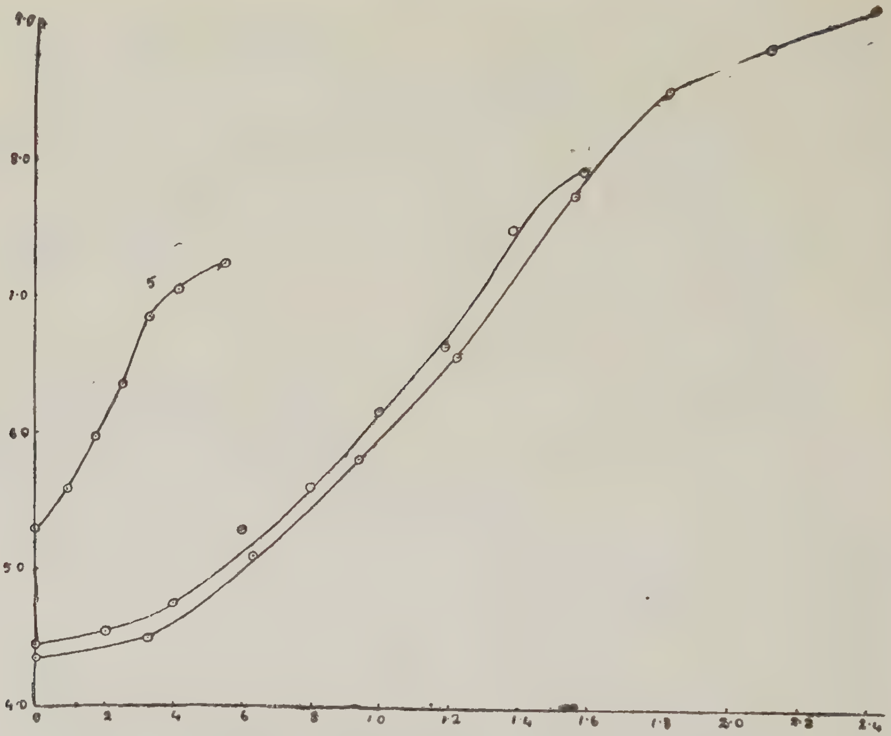
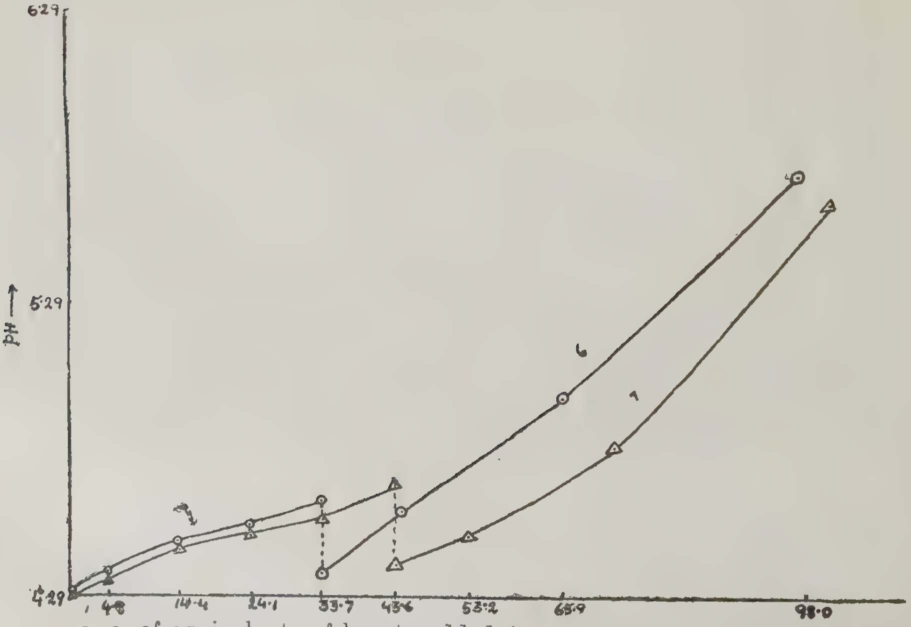
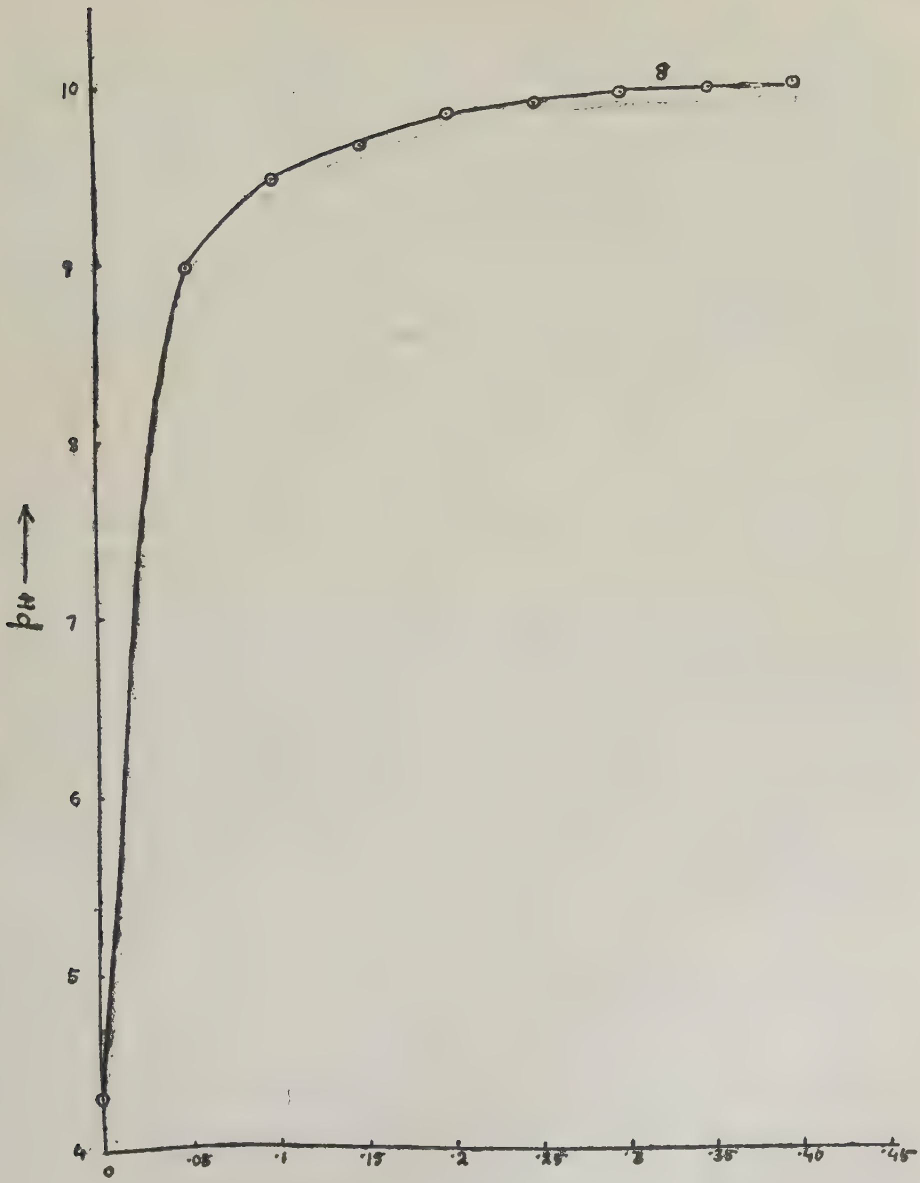
* More detailed investigations in this direction are in progress.



c. c. of 0·0063 *N* baryta added to 25 c. c. of sol.
Fig. 2. Sol A.



c. c. of equivalents of baryta added (to 25 c. c. of sol) × 10^7.
Fig. 3. Sol A (Ganges sediment).



c. c. of strong NaOH (1·18N) added to 30 c. c. of sol.

Fig. 4. Sol A.

The low ratio of free to total acid of the sol indicates a weak acid character. The accuracy of the absolute value of this ratio is somewhat ill defined in view of uncertainties of absolute measurements with the hydrogen electrode with unbuffered solutions of extremely low hydrogen-ion concentration, especially in the absence of independent measurements with the quinhydrone electrode. Further, the error of the e. m. f. determination may be multiplied several times in calculating the hydrogen-ion concentrations from the pH values as it depends on the magnitude of the mantissa in converting it into its antilog.

In the titration of the acid clays with alkalis the time allowed for interaction has been found to be a very important factor. It has been repeatedly observed that on the addition of a given amount of alkali after the electrode has been in equilibrium with the sol to be titrated, the e. m. f. of the hydrogen electrode at first rises to a maximum and then diminishes very slowly till equilibrium is reached. This behaviour is observed near about the neutral region and it becomes pronounced in the alkaline region ; a slow adsorption of the alkali by the particles probably takes place ; the need of vigorous shaking during the titration is obvious.

The effect of the above slow reaction between the acid clay suspension and the alkali in which the alkali appears to disappear becomes more pronounced when a titration is continued for several days. It has been repeatedly observed that the initial pH on any day is lower than the final equilibrium pH (after the titration had been carried to a definite point) on the day previous to it. Curves 6 and 7 (Fig. 3) illustrate this point with Sol A_1. This observation is in contrast with our experience with silicic acid sols where, under similar circumstances, a slight increase in the initial pH on successive days has been observed. The effect of the above slow reaction has to be considered in the interpretation of the results of a titration where a second addition of the alkali was made when the e. m. f. of the hydrogen electrode corresponding to the previous addition did not change in half an hour's time.

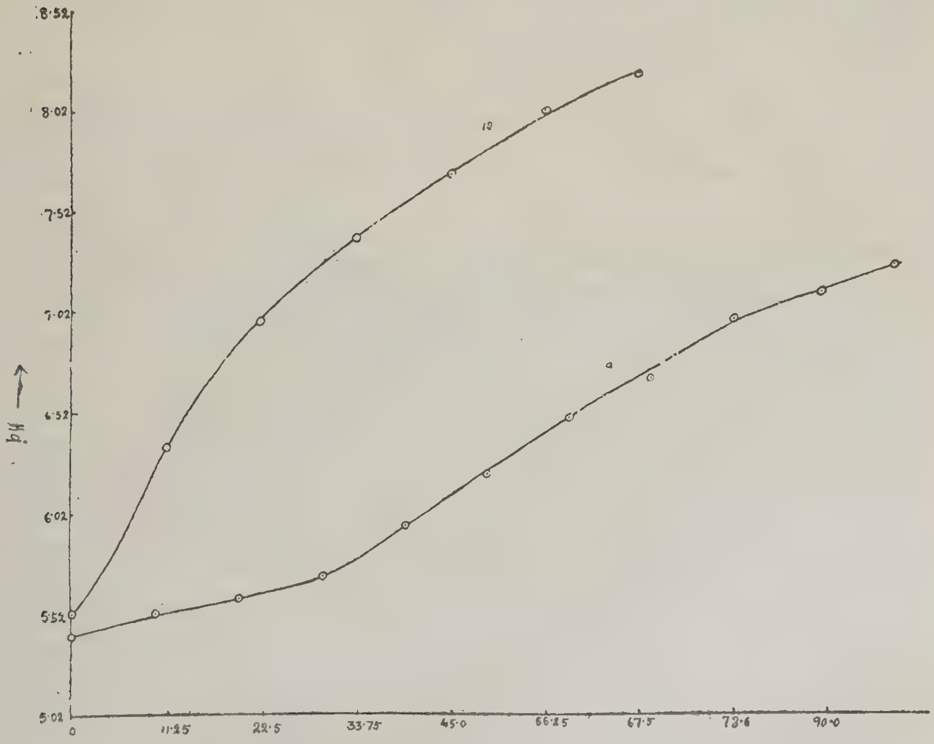
SOLS B AND B_1

The results with these sols are summarised below :

TABLE III

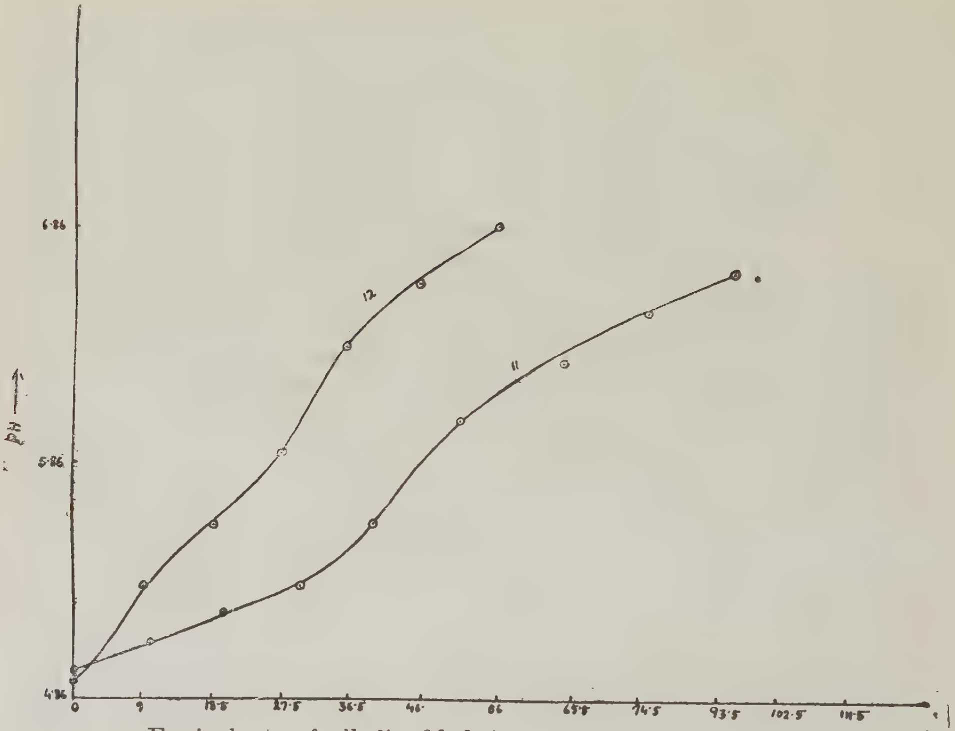
	Total acid × 10^5 in normality	pH (Average)	Free acid in normality
Sol B	18·0 (With Ba $(OH)_2$; curve 9) 7·0 (With NaOH ; curve 10)	5·29	$6{\cdot}0\times10^{-6}$
Sol B_1	15·0 (With Ba $(OH)_2$; curve 11) 6·6 (With NaOH ; curve 12)	4·92	$1{\cdot}2\times10^{-5}$

The titration curves with dilute alkalis resemble those of moderately strong acids ; with concentrated alkalis a weak acid character is indicated (compare sol A_1), the particles coagulating at the higher alkaline regions. The titration curve with barium hydroxide is different from that with caustic soda—a strong buffer action being observed with barium hydroxide. This observation is in agreement with the results of previous investigators with clay acids. Unpublished work from this laboratory with palmitic acid sols also point to a difference in total acidity on titration with different strong bases. These results support Mukherjee's previous conclusion based on theoretical considerations regarding the indefiniteness of the total amount of the neutralisable acid in colloidal acid systems [Mukherjee *et al.*, 1934]. As pointed out by Mukherjee the characterisation of the intensity factor of such acids in terms of the mass action expression becomes ambiguous because of the above indefiniteness of the total acid.



c. c. of equivalents of alkali added (to 25 c. c. of sol) × 10^7.

Fig. 5. Sol B.



Equivalents of alkali added (to 25 c. c. of sol) $\times 10^7$.

Fig. 6. Sol B.

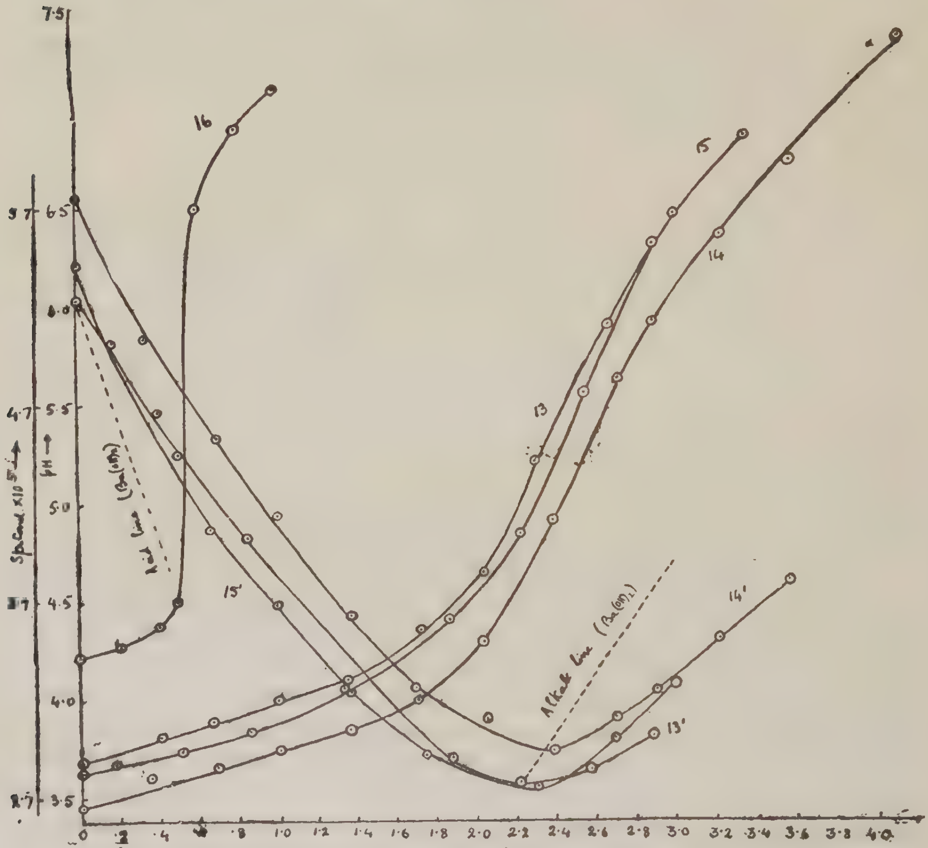
Sol C

In the preparation of sols A_1, B and B_1 a preliminary leaching with hydrochloric acid had been taken recourse to. The acid character of the sols might be attributed to hydrochloric acid adsorbed by the particles. It was therefore considered desirable to prepare an acid clay sol by electrodialysis alone omitting the hydrochloric acid treatment. Sol C was prepared in this manner after removal of organic matter by the usual treatment with six per cent hydrogen peroxide. The electrodialysis was effected in glass vessels with parchment membranes and platinum electrodes. The results are given in Table IV.

TABLE IV

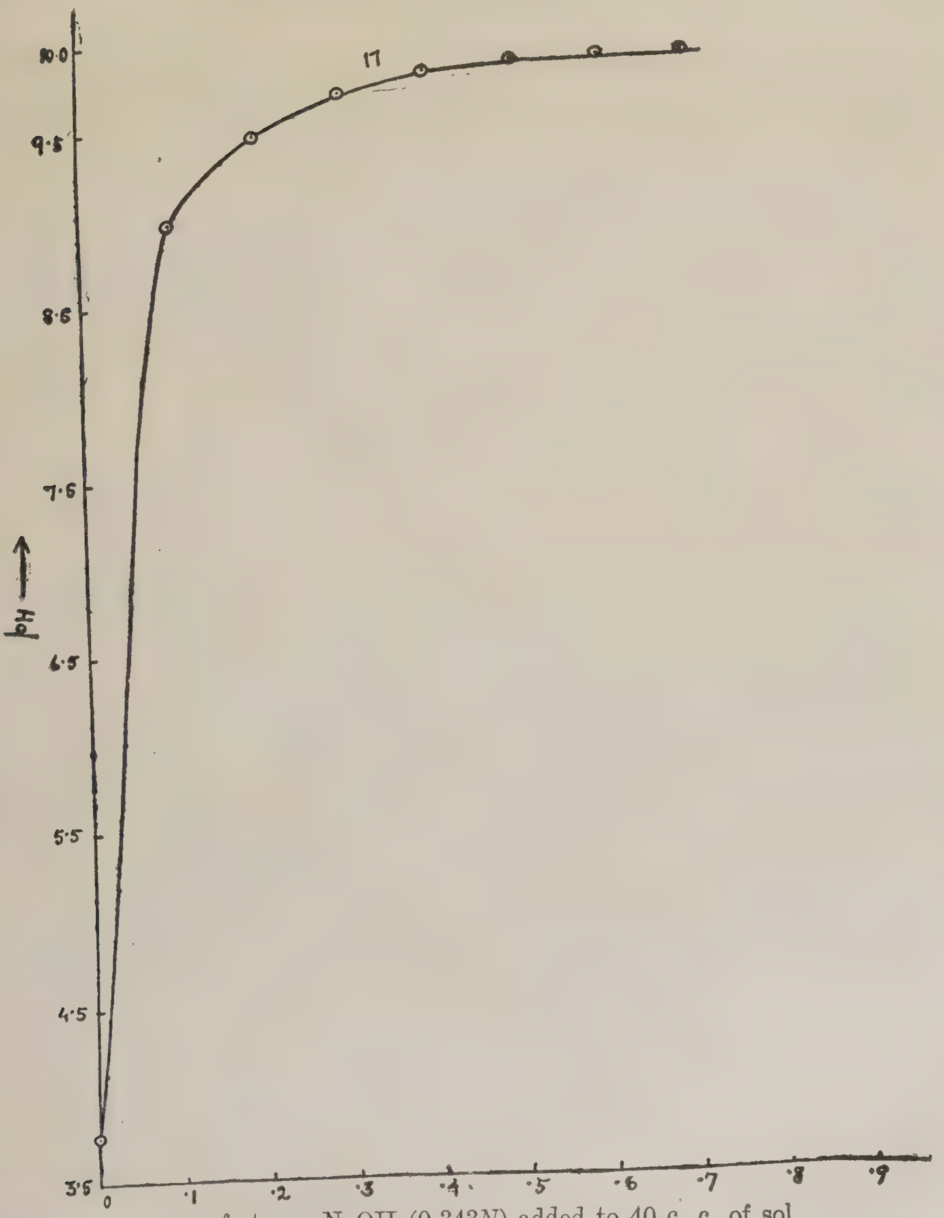
	Total acid $\times 10^4$ in normality	pH (average)	Free acid $\times 10^4$ in normality	$\frac{\text{Free acid}}{\text{Total acid}}$	Chlorine-ion conc. $\times 10^5$ in normality
Sol C . .	2·8 (curves 13, 14, 15 & 13′, 14′, 15′)	3·58	2·8	1·0	9·4
Ultrafiltrate of Sol C	0·7 (curve 16) .	..	..	..	9·5

The sol is distinctly more acid than any of the previous sols. The titration curves 13, 14, 15 and 13′, 14′, 15′ (Fig. 7) indicate a fairly strong acid character which is confirmed by the agreement between the free and total acids. The titration curve 17 (Fig. 8) with strong alkali indicates however, a weak acid character, though the final rise after a flat portion is not in evidence. The total acids calculated from the potentiometric and conductometric titration curves are identical and in contrast with the previous sols is the same with sodium and barium hydroxides. The conductometric titration curves on closer examination suggest a slow reaction with the alkali near the point of neutralisation. A comparison of the slope of the experimental curve with that of the theoretical alkali line indicates that the above slow reaction, involving probably an adsorption of the hydroxyl ions by the colloidal particles, is continued even after the minimum point has been passed. The acid clay sols show a striking similarity with colloidal solutions of silicic acid in this regard. The chlorine-ion concentration of the sol calculated from the e. m. f. of the cell Ag-AgCl/0·01 N HCl/Sol KNO_3/Sol/AgCl-Ag agrees with that of the ultrafiltrate obtained in an analogous manner. The small chlorine-ion concentration shows that the large free and total acids of the sol cannot be wholly attributed to free hydrochloric acid. The total acid of the ultrafiltrate is about twenty-five per cent of the total acid of the sol. The acidity of the ultrafiltrate is probably due to free hydrochloric acid as is shown by the approximate agreement between the total acid and the chlorine-ion concentration of the ultrafiltrate. Further, the potentiometric titration curve 16 of the ultrafiltrate is characteristic of a very strong acid. As the preliminary hydrochloric acid treatment was omitted in the preparation of the sol it is interesting to find the presence of free hydrochloric acid in the sol.



c. c. of 0·0064N alkali added to 50 c. c. of sol.

Fig. 7. Sol. C.



c. c. of strong NaOH (0·343N) added to 40 c. c. of sol.

Fig. 8. Sol C.

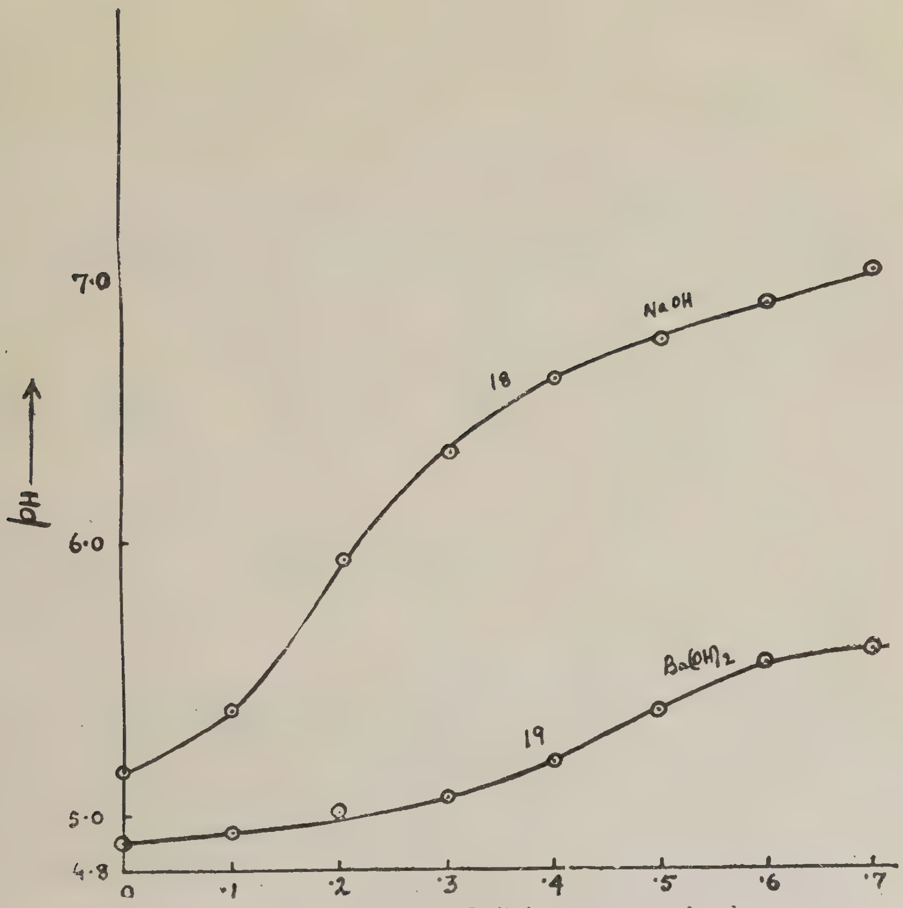
Sol C_1

This sol was isolated from the same soil sample as sol C but in preparing it the electrodialysis was preceded by a preliminary leaching of the colloidal material with 0·05 *N* hydrochloric acid. The results are given in Table V.

TABLE V

	Total acid	pH (average)	Chlorine-ion conc.
Sol C_1 . . .	$1{\cdot}91 \times 10^{-5}$ *N* (with NaOH ; curve 18). $5{\cdot}7 \times 10^{-5}$ *N* (with $Ba(OH)_2$; curve 19).	5·05	*Nil*
Ultrafiltrate of sol C_1	..	6·4	*Nil*

The acid character of clay acid appears to depend, among other factors, on the method of preparation of the sol. The difference in the electrochemical behaviours of sols C and C_1 isolated from the same soil sample illustrates this point. Sol C is definitely more acid than sol C_1. Unlike sol C the titration curves 18 and 19 (Fig. 9) of sol C_1 with sodium and barium hydroxides show a difference ; also the total acid is greater than the free acid. Sol C is a dilute sol containing 0·44 grm. of the colloid per litre while the colloid content of sol C_1 is 0·72 grm. per litre. Also while sol C is practically free from iron, sol C_1 contains eighteen per cent ferric oxide in terms of the oven-dried residue. This difference in ferric oxide content probably arises from the fact that whereas sol C_1 had been electrodialysed after a preliminary leaching with dilute hydrochloric acid sol C was electrodialysed directly. The above difference in composition of the colloidal material is probably responsible for the incongruous features presented by the two sols.



c. c. of 0·0064*N* alkali in 50 c. c. of sol.

Fig. 9. Acid clay sol C_1.

Neutral salts liberate (see later) considerable amounts of acid with both the sols. In view of the practically complete agreement between the free and total acidities of sol C, it is most interesting to note the liberation of acids by neutral salts from this sol. This observation is of fundamental importance in relation to the theoretical treatment of the proporties of colloidal acids.

LIBERATION OF ACIDS BY SALT SOLUTIONS

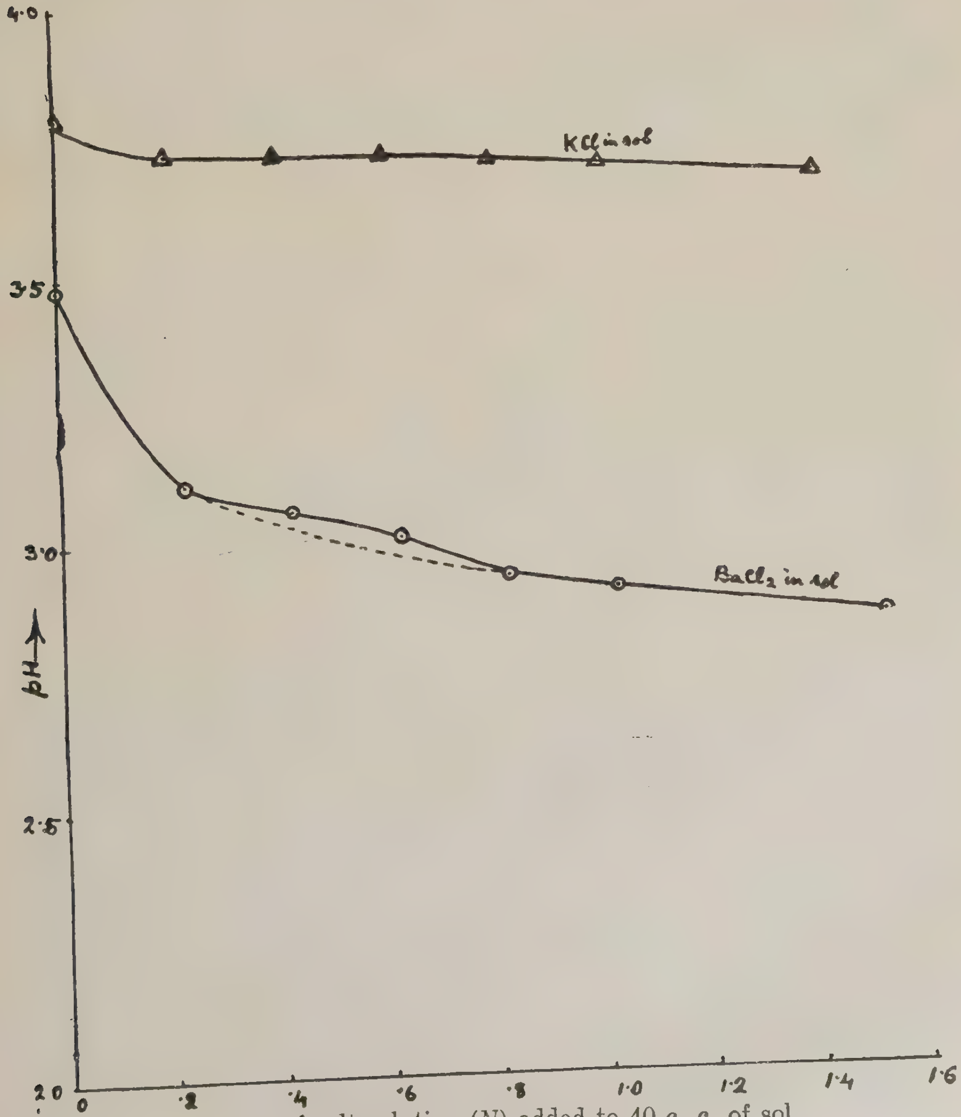
Titrations of colloidal clay acids by salt solutions are of interest as throwing light, among other things, on the mechanism of the phenomena of exchange acidity of soils. Systematic investigations are necessary to understand the exact nature of the reactions involved. The results of experiments in this direction are given below:

	Total acid obtained by titration with alkalis without addition of salt	Initial pH (without salt)	Conc. of salt added	pH after addition of salt	Curve number
Sol C	$2{\cdot}8 \times 10^{-4}$ *N*	3·48	0·037*N* $BaCl_2$	2·85	20
	Do.	3·80	0·034*N* KCl	3·67	21
Sol C_1	$1{\cdot}91 \times 10^{-5}$ *N* (with NaOH)	5·13	0·006*N* $BaCl_2$	4·58	22
	$5{\cdot}7 \times 10^{-5}$ *N* (with $Ba(OH)_2$).	5·05	0.006*N* KCl	4·85	23

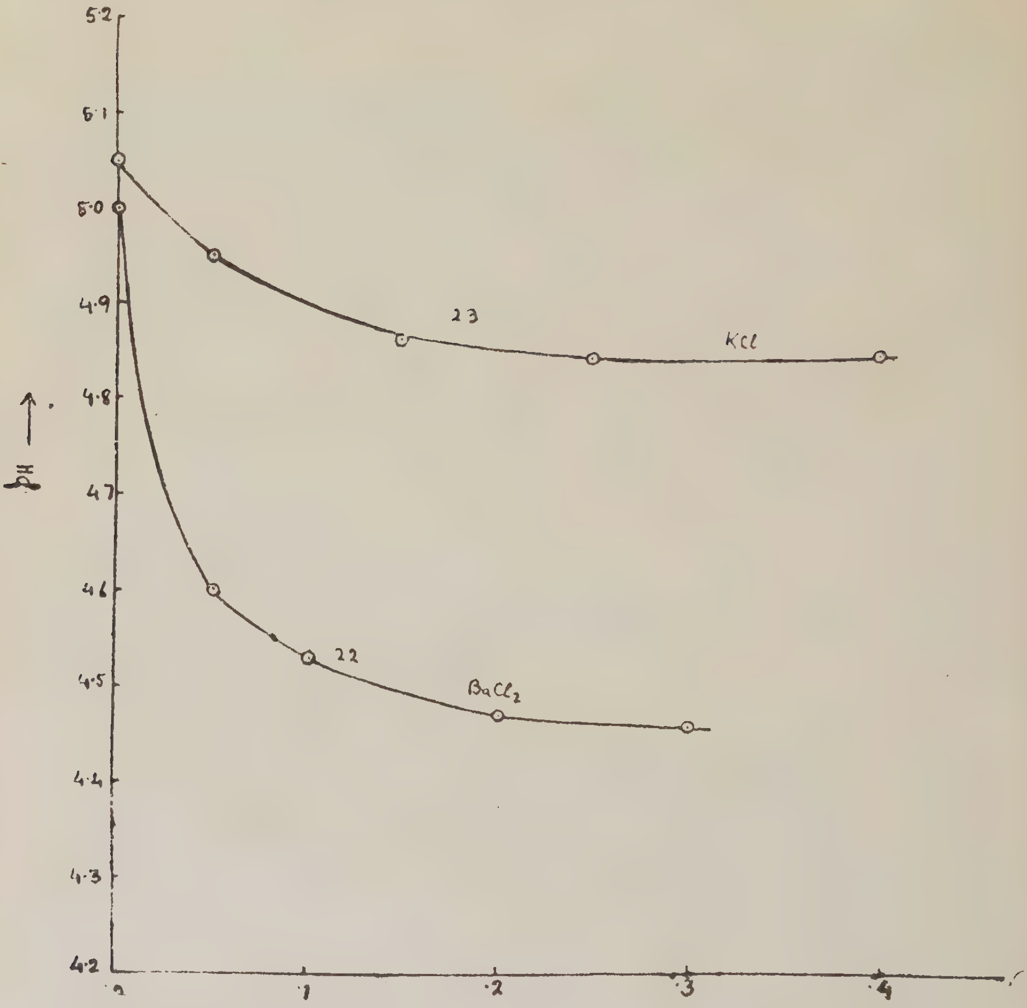
It will be seen that neutral salts liberate considerable quantities of acids from the clay acids—barium chloride being more powerful than potassium chloride in this respect [Mukherjee *et al.*, 1936]. As already stated the liberation of acids in the case of sol C is particularly interesting in view of the apparent strong acid character of the sol as indicated by the form of its titration curves as also by the agreement between the free and total acids.

According to Mukherjee [1922] the liberation of acids by neutral salts arises from an interchange between the cation of the added electrolyte and the hydrogen-ions electrically adsorbed on the surface of the colloidal particles. Exchanges with such cations will not alter the observed hydrogen-ion concentration of the medium so long as only the mobile hydrogen ions interchange their places with the cations in the bulk of the solution. The osmotic penetration of the cations into the double layer may, under favourable circumstances, produce a shift in the distribution of the hydrogen ions between the primarily adsorbed layer and the mobile layer, and thus produce a variation in the observed hydrogen-ion activity. The interchange is governed by the relative adsorbabilities of the cations depending on their valencies, mobilities and states of hydration. One thus obtains the well-known valency series when electric adsorption alone determines the adsorbability. It is interesting to find that in all the above cases the concentration of hydrogen-ions in displacement equilibrium with different salts is in agreement with the above series. Barium chloride in all cases liberates more acid than the alkali metal salts.

The liberation of acids by neutral salts in the case of colloidal acids which are apparently completely dissociated (*e.g.* acid clay sol C) as is shown by the equality of their free and total acids is difficult to understand in the light of classical electrochemistry as such acid liberation is tantamount to an incomplete dissociation.



c. c. of salt solution (*N*) added to 40 c. c. of sol.
Fig. 10. Acid clay sol C.



c. c. of N salt solution added to 40 c. c. of soi.
FIG. 11. Sol C.

BASICITY OF ACID CLAY SOLS

Colloidal clay acids have often been looked upon as polybasic acids in true solution. The titration curves given in this paper bear no evidence of the different stages of dissociation characteristic of polybasic acids and are typical of monobasic acids. The alkaline regions of the curve also resemble those of monobasic acids and the adsorption of alkalis by the colloidal particles is sufficient to account for their form. In agreement with the observation of some previous investigators regarding stages of dissociation of soil acids sudden changes in the slopes of the titration curves have sometimes been observed in this laboratory but they disappeared when the colloidal solution was stirred vigorously during the titration. These sudden changes simulate the stages of dissociation of a polybasic acid and arise out of local increases in the concentration of the alkali which has not mixed thoroughly with the whole liquid.

THE INTENSITY FACTOR OF SOIL ACIDITY

While several methods exist for determining the total acidity and the degree of saturation of soils, it has not yet been possible to obtain an estimate of the intensity factor of the acidity. If the clay acids could be treated as acids in true solution, titration curves would afford useful information regarding the total acidity and the intensity factor expressed as the dissociation constant. The foregoing results and also previous work from this laboratory show that the total amount of acid taking part in the interaction with alkalis is a variable quantity depending, among other things, on the nature and concentration of the alkali and on the extent of dispersion of the acid substance and is not simply determined by its total concentration. The interaction involving the interface constitutes the difference.

The conception of a secondary ion adsorption (electrical and chemical adsorption) put forth by Mukherjee offers a better understanding of the characteristic behaviour of colloidal acids. According to this view a colloidal acid clay particle owes its charge (negative) to the presence on its surface (by primary adsorption) of a fixed layer of ions. Corresponding to the number of these ions an equivalent number of oppositely charged ions remain in the liquid near the surface. A layer of oppositely charged ions may remain fixed on the surface by electrostatic and valence forces thus forming an osmotically inactive secondarily adsorbed layer on the surface. As already stated the liberation of acids by neutral salts is, on the above view, brought about by the interchange between electrically adsorbed hydrogen ions and the cations of the added electrolyte. The difference in the forms of the titration curves with sodium and barium hydroxides results from a stronger adsorption of the barium ion. The buffer action, particularly with barium hydroxide, in the acid region indicates the possibility that the surface molecules of the acid which are ' built into ' the crystal lattice of the particles may have a stronger acid character than those in true solution.

According to the above view the nature of the primarily adsorbed ions and their state of adsorption determine the ' strengths ' of colloidal acids. Experimental methods throwing light on the mechanism of interchanges between the ions in the ' double layer ' and the ions in the intermicellar liquid can be outlined. Thus the necessary information regarding the condition and the nature of the primarily adsorbed ions may be obtained. These aspects of the problem will be later more fully discussed.

SUMMARY

1. The electrochemical behaviours of several acid clay sols isolated from a number of soil samples with the aid of Sharple's Supercentrifuge have been investigated. The intensity of the acid character of these sols have been discussed with reference to the nature of their titration curves with different strong bases and with different concentration of alkalis.

2. The initial pH values calculated from the e. m. f.'s of the hydrogen electrode sometimes show wide variations due, probably, to a poisoning of the hydrogen electrode. The form of the titration curve is, however, very little affected by a ' poisoned ' electrode and the total acidity of a given sol calculated from different curves show satisfactory agreement inspite of appreciable initial differences of the curves.

3. Titration curves with dilute alkalis point to a moderately strong acid character. With concentrated alkalis a weak acid character is indicated. The curves are remarkably smooth. No evidence of stages of dissociation are obtained.

4. The titration curves give evidence of reactions distinct from a simple neutralisation of hydrogen by hydroxyl ions. The curvature of the conductometric titration curves in the acid region and near about the endpoint, point to an adsorption of the alkali by the interface.

5. The total acid calculated from the titration curves is different with different strong bases. With concentrated alkalis the particles coagulate in the alkaline region and the titration curves simulate those of weak acids with the difference that the final rise after a flat portion is not observed even when the alkali added would neutralise several times the total acid calculated from the titration curves with dilute alkalis. The total acid is thus an indefinite quantity.

6. Neutral salts liberate considerable amounts of acids from the sols—the alkaline earth cations being more effective in this respect than the alkali metal cations. The effect of addition of neutral salts has been discussed from the point of view of electrical adsorption of ions.

7. The results indicate certain characteristic behaviours of the clay acids and en-phasise the need of a theoretical treatment of these acids which must differ from that of acids in true solution.

The author's thanks are due to Professor J. N. Mukherjee, D.Sc. for his kind interest in the work ; to the University of Calcutta for permission to work in the

University Science College laboratories and for other facilities and to Mr. M. C. Carbery, M.A., B.Sc., Agricultural Chemist to the Government of Bengal for supplying the soil samples. The author is grateful to the Imperial Council of Agricultural Research, India, for a grant which enabled him to carry out this work and for other facilities.

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INDIAN SOIL FUNGI

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(Received for publication on 19th February 1936)

In the earlier days of soil microbiology, attention was focussed almost exclusively on the bacteria present. It is now realised that saprophytic moulds and other fungi are also of great importance in soil processes. They are, for example, the principal agents in the decomposition of cellulose and hemicelluloses of dead plant material, and are capable of carrying out their activities with lower moisture requirements than are the bacteria. Certain soil fungi as well as soil bacteria are capable of producing ammonia from the protein of plant and animal debris, thus assisting to make the organic nitrogen once more available for plant growth, although the later stages of conversion are left to the nitrite- and nitrate-forming bacteria. Conversely, nitrates and ammonium salts in the soil are converted by soil fungi into organic nitrogen, "locked up" in the fungal tissues, and only available to crop plants after the breakdown of those tissues.

Another important aspect of soil saprophytes is the part they play in suppressing potential plant pathogens present in the soil. This may occur simply because they are better adapted to a saprophytic life and are thus able to starve out the parasitic fungi ; or, as has been shown for certain instances [Weindling, 1932], the saprophyte may produce a definite toxin.

A complete study of the fungus flora of soil presents many difficulties. Not all the organisms present are capable of growing on artificial media, and many of the pathogenic fungi can only be detected by their effect on crop plants. The fungi that develop on laboratory media are the saprophytes and facultative saprophytes, the majority of which belong to the difficult group of Fungi Imperfecti. Identification is a matter of considerable difficulty, and no "host index" can be consulted as in the case of a plant parasite.

Owing to the fact that fungi exist in the soil both as mycelium and spores, the numbers of colonies obtained on dilution plates of a soil sample by no means indicate the relative importance of these organisms in the soil. A cluster of dormant spores will produce many colonies, whereas a mass of active mycelium may give only one.

Bacteria in the soil so out-number the fungi that precautions have to be taken to suppress bacterial growth—if possible without affecting fungal growth. A fairly close approach to this can be made by slightly acidifying the medium to a degree that checks bacteria but that has little effect on the majority of soil fungi.

Subject to these limitations, the "plating out" technique gives a useful indication of the types of saprophytic fungi present. It may be supplemented by "direct examination" of mycelium in the soil, and by "direct isolation" of the mycelium that is first put out from particles of soil placed on an agar medium and incubated. Surveys of soil fungi have been carried out in various countries, and the most striking point brought out is the universal distribution of the commoner soil organisms. This is a point of economic interest, for most "air infections" are really soil organisms accompanying dust particles; hence the moulds causing decay of foodstuffs, textiles, and similar industrial and agricultural products show a remarkable similarity all over the world, and uniform methods of control can be adopted. The work of Dale [1912, 1914], and Brierley [1923] in England; of Jensen [1912], Waksman [1916, 1917], Werkenthin [1916], Gilman and Abbott [1927], and LeClerg [1931] in America; Bisby and his co-workers [1933, 1935] in Canada; Oudemans and Koning [1902] in Holland, and Jensen [1931] in Denmark, may be mentioned, and a good summary of the earlier work is given by Waksman [1932].

In India, little attention has been given to soil fungi. It is very desirable that the soil flora of this country should be more extensively studied; as, however, it is not clear that such a study would lead to immediate practical results, this work is perhaps more suitable to the Universities than to research workers of the agricultural departments. Shaw [1915] records four isolations from Pusa soils; Thakur and Norris [1928] describe about twenty-five species from Madras soil, with special reference to their power of cellulose decomposition and ammonification; Mason [1928] mentions five species isolated by Rhind in Burma; and finally Chaudhuri and Sachar [1934] give full descriptions of thirty-two species isolated from soil samples taken at Lahore.

TABLE I

Soil samples for isolation of fungi

No.	Source	Date	Moisture per cent	pH
	Alkaline soils			
1	Pusa Garden soil	Feburary .	6·5	8·5
2	,, ,, ,, ...	September .	16·0	7·9
3	,, Experimental plot . .	March . .	4·5	8·0
4	,, ,, ,, . .	September .	15·0	8·3
5	,, Kitchen garden . . .	March . .	13·8	8·5
6	,, ,, ,, . . .	October .	13·7	8·05
7	,, Low land by river . .	March . .	18·3	8·1
8	,, ,, ,, . . .	November .	..	8·4
9	,, Permanent manural plot .	April . .	7·4	...
10	,, ,, ,, ,, .	October .	11·8	8·15
11	Karnal	February .	11·4	7·8

TABLE I—*contd.*

Soil samples for isolation of fungi

No.	Source	Date	Moisture per cent	pH
	Neutral soils			
12	Karnal	October .	9·6	6·9
13	Simla	August .	23·5	6·9
	Acid soils			
14	Darjeeling (1″-4″). . . .	June . .	. .	6·3
15	,, (6″-8″)	,, . .	. .	. .
16	Mungpoo	August .	40·0	6·1
17	Ramgarh	,, .	33·0	6·5
18	Chaubattia	,, .	6·0	6·5
19	Kulu	,, .	10·5	6·5
20	Manali	,, .	23·5	6·3

EXPERIMENTAL

The present account summarises a very hasty survey of a number of soil samples taken in 1935, mainly from Pusa and from certain hill districts in Northern India. The sources from which samples were taken are shown in Table I, and from the first six of these two samples were taken, one in early summer and one in the wet season.

Method of sampling and dilution.—Samples were removed by means of a copper cylinder measuring six inches in length and one inch in diameter, sharpened at one end and extended into a handle at the other. Several such cylinders were made and sterilised for use as required. When a soil sample was to be taken, the loose surface soil was pushed aside, and a plug taken of the top 4—6 in. ; this was repeated from six places a foot apart and the plugs of soil placed in a sterile stoppered jar. (Samples of soils 13—20 were taken with an ordinary large spatula). At the earliest opportunity, the contents of the jar were rubbed through a sterile sieve of wire mesh, well mixed together, and after removal of a portion for moisture determination, returned to the stoppered jar.

The moisture content having been determined, the equivalent of twenty-five grams of dry soil was weighed out and transferred to a one-litre Roux flask containing 250 c.c. of sterile tap water. The flask was then fitted with a rubber stopper—dipped in alcohol and flamed—and the contents shaken steadily for ten minutes. Three successive ten-fold dilutions were made by transferring with sterilised 10-c.c. pipettes to three lots of 90 c.c. of sterile water contained in 250-c.c. flasks. The second and third of the small flasks thus represented dilutions of 1 : 1,000 and 1 : 10,000. Duplicate one-c.c. samples of each of these dilutions were placed in Petri dishes and the agar medium added after cooling in the usual manner. The plates were inverted when set, and incubated at 25°C. In the absence of an available cool incubator, some of the earlier samples rose to summer laboratory temperature, which occasionally reached 35°C.

Media.—The medium principally employed was that recommended by Bisby [1933], *i.e.*, Dox's agar acidified by the addition of 0·5 c.c. of 12·5 per cent lactic acid to each 100 c.c. medium. The acid was sterilised separately, and added at the time of plating. This medium suppresses bacterial growth very effectively, and is convenient to prepare and use. For certain fungi, such as the Mucors, however, a sucrose medium is not suitable ; duplicate platings were therefore made on an alternative medium which was also acidified. At first malt extract agar was tried for the purpose, and later Quaker oat agar was used. Attempts to find a substitute for acidity in suppressing bacterial growth—such as the employment of a medium of high sugar content—proved unsuccessful.

Counts.—Approximate counts were made on the eight plates from each soil sample, and as a rule these fell consistently between 20,000 and 40,000 per gram. The only variation was in samples 14 and 15, where the soil just below the surface gave 40,000—50,000, and the deeper soil only 10,000—20,000 per gram. This is in agreement with usual experience that fungi are most plentiful in the top few inches of soil.

TYPES OF FUNGI ISOLATED

The fungi recorded below represent those types which occurred most frequently on the plates. It must again be emphasised that they represent only a portion of the total fungi present. Soils 11 and 12, for example, were known to contain *Rhizoctonia*, but no colony of this appeared on the plates. Mucors were infrequent, although the technique of "direct isolation" from particles of soil would doubtless have yielded more. No Basidiomycetes were recorded.

Isolations have been allotted to the correct genus whenever possible. Pending further data as to which soil types are really widespread in India, and which are merely fortuitous, species determination has not been pressed beyond the limits to which the general mycologist can safely go, without reference to specialists. At a later date, when more material is available, it may be possible to enlist specialist aid in the identification of *Penicillium* and *Fusarium* spp. isolated from Indian soils.

From the present samples, the genera most frequently isolated were *Aspergillus* and *Penicillium*, the former being predominant in the Pusa soils. Several workers, *e.g.*, Waksman [1917], have pointed out that *Aspergillus* is more abundant in warmer climates and *Penicillium* in cooler ones. Of the thirty-two Indian soil fungi described by Chaudhuri and Sachar [1934] twelve belong to the genus *Aspergillus*. In the following list the classification of Thom and Church [1926] is adopted.

Asp. nidulans Eidam occurred most abundantly, being found in samples 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15. The isolations differed considerably, but all produced abundant perithecia, containing ascospores (rough in some isolations, smooth in others) with a double equatorial frill. On mounting the perithecia in lactophenol and pressing gently on the coverslip, a characteristic purple colour is given by the spores. This species is recorded in Indian soils by Chaudhuri and Sachar, and by Thakur and Norris.

Asp. niger v. Tiegh. comes next in order of abundance, in soils 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 14. It has been recorded by Shaw, Thakur and Norris, Mason, and Chaudhuri and Sachar.

Asp. terreus Thom, a sand-brown form, was isolated from samples 1, 2, 3, 7, 8, 10, 11. It is recorded by Mason, and by Chaudhuri and Sachar.

Asp. ustus Bain. Isolations of a dark brown mould from samples 3, 6, 7, 9, 10 and 15 differed among themselves, but agreed most closely with this species.

Asp. fumigatus Fres., one of the most abundant species in most soils, was unexpectedly absent from all the plates made from alkaline soils, although recorded from Pusa by Shaw [1915]. It occurred, however, in samples 16, 18 and 19. It is also recorded by Chaudhuri and Sachar, and by Thakur and Norris, although the latter state that it has no cellulose decomposing activity, a statement that throws some doubt on their identification.

Other species of *Aspergillus* were less abundant than those already named ; *Asp. flavipes* B. et S., from samples 1 and 2, is recorded by Chaudhuri and Sachar ; *Asp. flavus* Link. (samples 5, 11, 15) by Thakur and Norris ; *Asp. sydowi* B. et S. (sample 7 only) by Chaudhuri and Sachar ; *Asp. tamarii* Kita (sample 3) by Mason ; while *Asp. ochraceus* (sample 5), and *Asp. chevalieri* Mang. (samples 7 and 15) do not appear to have been previously recorded for India. Three isolations of *Aspergillus* spp. (from soils 11 and 15) have not been identified.

Penicillium.—This genus was present in most of the soils, but was far more abundant in the acid soils from hill districts. Probably the lower temperature was the chief factor concerned.

The systematy of *Penicillium* is still far from satisfactory, and exact determination of even the well-known species is a matter of great difficulty ; beyond these are several hundred species or strains all regrettably similar in their morphology. For the present, the soil isolations recorded here are merely allotted to their main groups.

Monoverticillata from samples 1, 7, 10, 11, 13, 15, 16 and 17.

Biverticillata symmetrica from samples 10, 13, 14, 15 and 19, all giving purple or orange pigment.

Unclassified from samples 2, 3, 8, 11, 15, 17 and 19.

The remaining isolations, of the less abundant genera, may now be taken in their systematic order.

PHYCOMYCETES

The Mucoraceae were comparatively infrequent on the plates made, and are probably less abundant than in temperate climates. The following types were found :—

Rhizopus arrhizus Fischer, from samples 3, 6, 9, 13 and 17. This is closely related to *Rhiz. nigricans* Ehrenb., but is somewhat lighter in colour,has less well-developed rhizoids, and is capable of growth at a higher temperature. It is recorded by Chaudhuri and Sachar.

Mucor spp. from samples 10 and 14.

Cunninghamiella sp., probably *C. echinulata* (Thaxt.) Sacc. was obtained from sample 1. *C. elegans* Lend. is recorded by Shaw.

Syncephalastrum sp. Single colony isolated from sample 14. This may have been an atmospheric contamination. (*Syncephalis* sp. was isolated from manure and may also be regarded as a potential soil organism in India.

ASCOMYCETES

Perithecial strains of *Aspergillus* and *Penicillium* have already been dealt with. The only other *Ascomycetes* were two genera of the Sordariaceae and *Byssochlamys* sp.

Chaetomium ? indicum Corda from samples 5, 9 and 10.

Sordaria sp. from sample 2.

? *Byssochlamys* sp. On plates from samples 5 and 16 there occurred a whitish growth, later producing ochre coloured masses of conglomerate asci each containing a varying number of spores 2·5-3·5 × 2·5-3 μ No conidial stage was found.

FUNGI IMPERFECTI

The majority of genera isolated from soil belong to this group. Bisby [1933] states that of his isolations from Manitoba soils, over ninety per cent were Fungi Imperfecti.

Phomaceae

Phoma sp. was obtained from sample 5.

Melanconiaceae

Melanconium sp. from samples 5 and 7. Black spores 8-12 μ × 3·0-3·5μ. Waksman [1916] reports a species of *Melanconium* from soil with spores 6·2-8·6 × 2·5-2·8μ ; as in the present case, this species was found to be an active cellulose decomposer.

Moniliaceae

Monilia spp. A number of isolations (samples 1, 2, 3, 9, 10, 11, 15 and 17) could only be grouped under this somewhat vague genus. The species from 1 and 11 agrees well with *Monilia koningi* Oud., which is recorded by Chaudhuri and Sachar.

Trichoderma lignorum (Tode) Harz. was abundant in samples 16 and 20. It was not isolated from any of the alkaline soils. It is, however, reported in alkaline Sahara soils by Killian and Feher [1935]. This organism has been shown by Weindling [1932] to be parasitic on *Rhizoctonia solani* and certain other soil pathogens.

Dematiaceae

?Botryotrichum sp. (samples 1, 2, 7 and 10). Brown hair-like sterile hyphae present ; conidia subhyaline, lens-shaped to spherical, 6-8×4-5-μ.

?Mesobotrys or *Chaetopsis* sp., from sample 11.

Cladosporium herbarum Link was found in samples 1, 3 and 11 and other unidentified species of *Cladosporium* (here used to include *Hormodendron*) from samples 1, 10 and 11.

?Epochnium sp. isolated from samples 10 and 11. Both hyaline and dematiaceous conidiophores. Some two-celled spores seen.

Alternaria. Samples 5, 7, 11 and 14 yielded species of this genus, all belonging to the " *Alternaria tenuis* " group.

Helminthosporium sativum P. K. and B. was found in sample 11, and *H. tetramera* in sample 2.

Acrothecium lunatum Wakker from samples 10, 11 and 19. Recorded from Indian soils by Chaudhuri and Sachar.

Torula sp. An isolation from sample 15 is provisionally placed here.

Trichosporium sp. (from sample 7 only). Spore measurements agree with *T. fuscum* (Link.)

Dematium sp. (samples 11 and 15).

Coniothecium spp. (samples 3, 7 and 10).

Coniosporium sp. from samples 2 and 9. Spores black, 10-12 μ.

Tuberculariaceae

Fusarium spp. were isolated from the following : 3, 11, 13, 15 showed macrospores ; in 2, 4, 11, 14 and 19 only microspores were seen and some of these species would perhaps be better classified as *Cephalosporium.* No one type appeared to be prominent, and the species were not identified.

Myrothecium sp. from sample 1. Black sporodochia ; conidia 7-8 μ×2·5μ greenish black. This is probably *M. roridum* Tode reported from American soils.

Stilbaceae

Stysanus sp. One species, agreeing with *S. stemonites* (Pers.) Corda was obtained from a sample of Pusa soil not shown in Table I.

Unidentified.—Owing to the absence of spore structures, or through loss of original cultures, twelve isolations (samples 1, 4, 11, 14, 15, 16 and 17) have not been even approximately identified. Most of these gave a sparse whitish growth on agar, and some were probably identical.

POWER OF DECOMPOSING CELLULOSE

Action on cellulose was tested by ability to grow on filter paper strips half immersed in tubes containing the following medium. Since the only carbon source was the filter paper, vigorous growth indicated power to attack cellulose, although the converse was not necessarily true in all cases.

Medium.—Aq. dest. 1,000 c.c. ; $NaNO_3$ 0·5 grm. ; K_2HPO_4 1·0 grm. ; $MgSO_4.7H_2O$ 0·5 grm. ; KCl 0·5 grm. ; $FeSO_4.7H_2O$ 0·01 grm.

The most active cellulose decomposes were : *Aspergillus fumigatus*, *A. ustus*, *Asp. terreus*, *A. ochraceus*, *Penicillium* spp. (mostly monoverticillate), *Chaetomium*, *Trichoderma*, *Melanconium*, *Alternaria*, *Acrothecium*, *Helminthosporium*, *Stysanus*, *Dematium*, *Trichosporium* and *?Epochnium*.

The following species grew on the filter paper, but less actively : *A. nidulans*, *A. flavipes*, *A. niger*, and *A. flavus* ; *Penicillium* spp., *Sordaria*, *Phoma*, *Monilia* spp., *?Byssochlamys*, *Fusarium* spp., *Coniothecium* and *Botryotrichum*.

SUMMARY

Two hundred isolations from Indian soils have been made and allotted to their approximate systematic position ; about thirty genera are represented.

The most striking feature is the predominance of *Aspergillus* spp. in most of the soils examined, and the limited distribution of such common soil types as *Aspergillus fumigatus* and *Trichoderma* spp.

The types most active in cellulose decomposition are recorded.

ACKNOWLEDGMENT

Considerable assistance was afforded by Mr. R. R. Sen in the examination of the cultures.

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STUDIES ON *SCHISTOCERCA GREGARIA* FORSK.

*IV. COLOUR CHANGES AND SEXUAL BEHAVIOUR IN DESEXUALISED *SCHISTOCERCA GREGARIA* ADULTS

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(Received for publication on 18th January 1936)

INTRODUCTORY

Many species of locusts exhibit more or less marked colour changes during the adult stage. Lefroy [1906] described these changes in *Patanga succincta*, Uvarov [1911] in *Locusta migratoria*, La Baume [1918] in *Dociostaurus maroccanus*. Künckel had previously described this phenomenon in *Schistocerca gregaria*. Uvarov [1928] has summarised these colour changes in the above species, and with regards to *Schistocerca gregaria* he says :—In this species the adults for about a month after reaching that stage are of a wine-rose colour, which gradually changes to a brownish yellow ; the intensity of the latter increases and fully mature individuals are of a brilliant yellow, the males being particularly brightly coloured, while the females are somewhat darker and have a greyish tint." According to Uvarov [1928] : " The extensive physiological changes that occur during the development of the genital products find their external expression in changes of coloration ". The fact that, as a rule, the sexual maturity can be determined by the intensity of yellowing lends support to this view.

The best method of studying the influence of the sex-glands, on the change of coloration, is to remove them and note the effect. This is the line along which we worked.

The great practical importance of the phenomenon of change of colour is evident, because the colour of the fliers in a swarm provides an index to the degree of " ripness " of locusts for copulation and oviposition.

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*Studies on *Schistocerca gregaria* Forsk,

I. The Micropyle in *Schistocerca gregaria* Forsk., and some other Acridiidae. *Ind. J. Agric. Sci.* **3** : 639-645.

II. The Biology of the Desert Locust with special relation to Temperature. *Ind. J. Agric. Sci.* **6** : 188-262.

III. Why Locusts Eat Wool ? A study in the hydromania of *Schistocerca gregaria*. *Ind. J. Agric. Sci.* **6** : 263-67.

This investigation formed part of the Locust Research Scheme financed by the Imperial Council of Agricultural Research, India. The generosity of the Council is gratefully acknowledged.

TECHNIQUE

A small slit was made, with a pair of fine scissors, in the pleural membrane of the 6th abdominal segment and the gonads removed by means of forceps. The instruments were properly sterilised. The opening was sealed with ordinary paraffin wax. It was found convenient to make this cut in the left side of the insect.

TABLE I

Longevity of desexualised males and females

Cage No.	No. of adults	Date of acquiring wings (1930)	Date of operation (1930)	Date of death (1930-31)	Total life in days	Remarks
			(A) *Overwintered brood (1930-31)*			
5	1 Male	4th Oct.	11th Oct.	20th Oct. 1930	16	No females were provided.
5	3 Males	Do.	Do.	27th Oct. 1930	23	
5	2 Males	Do.	Do.	3rd Nov. 1930	30	
5	1 Male	Do.	Do.	3rd Dec. 1930	60	
5	1 Male	Do.	Do.	31st Mar. 1931	178	
5	1 Male	Do.	Do.	8th April 1931	186	
6	1 Female	Do.	8th Oct.	9th Oct. 1930	5	No males were provided.
6	3 Females	Do.	Do.	10th Oct. 1930	6	
6	2 Females	Do.	Do.	27th Oct. 1930	23	
6	1 Female	Do.	Do.	17th Nov. 1930	44	
6	1 Female	Do.	Do.	19th Nov. 1930	46	
6	1 Female	Do.	Do.	29th Nov. 1930	56	
6	1 Female	Do.	Do.	26th Nov. 1930	83	
6	1 Female	Do.	Do.	29th Mar. 1931	176	
6	2 Females	Do.	Do.	5th April 1931	183	
6	1 Female	Do.	Do.	24th April 1931	202	
11	1 Female	16th Oct.	16th Oct.	9th April 1931	175	Castrated males were provided and copulation occurred.
19	1 Female	4th Oct.	17th Oct.	4th May 1931	212	
12	1 Female	16th Oct.	16th Oct.	9th April 1931	175	
34	1 Male	4th Oct.	11th Oct.	4th April 1931	182	Normal females were provided. Copulation and egg-laying took place.
35	1 Male	Do.	Do.	18th April 1931	196	
11	1 Male	Do.	19th Oct.	30th April 1931	208	Desexualised females were provided. Copulation occurred.
12	1 Male	16th Oct.	16th Oct.	20th May 1931	216	
14	1 Male	Do.	Do.	16th April 1931	182	

TABLE I—*contd.*

Cage No.	No. of adults.	Date of acquiring wings (1930)	Date of operation (1930)	Date of death (1930-31)	Total life in days	Remarks
			(B) *Spring brood* (1931)			
I 17	1 Female .	18th—25th May	31st May .	28th June . .	~~8±2~~	Normal male was provided on 2nd June 1931.
I 18	1 Female .	18th—25th May	Do. .	16th July . .	55±3	Normal male was provided on 2nd June 1931. Copulations were observed.
I 19	1 Female .	18th—25th May	Do. .	5th June . .	14±4	Normal male was provided.
I 19	1 Female .	2nd—8th June	10th June	5th Aug. . .	61±3	
I 23	1 Female .	18th—25th May	31st May .	18th June . .	28±3	Castrated male was provided.
I 20	1 Female .	20th May to 5th June	5th June .	22nd June . .	8±8	Normal males were provided.
I 20	1 Female .	21st June .	23rd June.	3rd July . .	12	
I 21	1 Female .	25th May to 5th June	5th June .	23rd July . .	56±8	
I 25	1 Female .	20th May to 5th June	8th June .	28th June . .	31±8	

TABLE II

Longevity of normal adults

Cage No.	Sex	Date of acquiring wings	Date of death	Total life (in days)
		(A) *Overwintered brood* (1930-31)		
		(1930)	(1931)	
39 a	Female	16th Aug. to 6th Sept.	13th April . .	230±10
47 a	Female . .	6th-7th Oct. . .	25th April . .	203±2
47 a	Male . .	2nd-7th Oct. . .	25th April . .	203±2
47 b	Female . .	Do. . .	13th April . .	191±2
47 b	Male . .	Do. . .	30th April . .	208±2
48 a	Female . .	Do. . .	19th April . .	197±2
48 a	Male . .	Do. . .	26th April . .	204±2
49 a	Female . .	27th-31st Oct. .	5th April . .	158±2
49 a	Male . .	Do. . .	26th Feb. . .	120±2
		(B) *Spring brood* (1931)		
Q 1	Female . .	11th-15th July .	11th Sept. . .	60±2
Q 1	Male . .	Do. . .	31st Aug. . .	49±2
II 5	Female . .	26th-29th June .	16th Aug. . .	51±2
II 5	Male . .	Do. . .	22nd Aug. . .	57±2
II 7	Female . .	12th-16th July .	30th Aug. . .	49±2
II 7	Male . .	Do. . .	6th Sept. . .	56±2
II 11	Female . .	16th-26th July. .	30th Sept. . .	76±5
II 11	Male . .	Do. . .	20th Sept. . .	66±5

All the operated males that had copulated were dissected at death and minutely examined. In no case was any trace of the testes found and we concluded from this that the removal was complete and the organ had not regenerated in any case.

LONGEVITY OF DESEXUALISED ADULTS

Table I gives data regarding the longevity of the adults which had been operated upon. It will be noticed that in the case of the overwintered brood about sixty per cent of the individuals died within the first two months or so of the operation, but the remainder survived for fairly long periods extending to 216 days. These figures compare very favourably with those of the longevity of the normal overwintering locusts of which a few representative cases are given in Table II.

Similarly, of the desexualised locusts of the spring brood, which were bred in the laboratory in 1931, about fifty per cent died within the first fortnight of the operation, while the remainder survived from twenty-five to sixty-four days. This again compares favourably with the longevity of normal locusts of the spring brood (Table II). It is evident, therefore, that the total extirpation of the sex-glands does not essentially interfere with the general life of the individuals.

COLOUR CHANGES

The overwintered insects, which had been operated upon, were greyish pink at the time of acquiring wings (October 1931), but as they advanced in age, they began to exhibit the characteristic colour changes. At first the bases of tegmina began turning yellow, and the deposition of the yellow pigments was then noticed on the abdominal segments. By about the middle of April most of the adults had developed bright yellow colour all over the body and this was particularly so in the male. This change in colour took place independent of the fact as to whether the adults were kept isolated (to simulate conditions of the solitary phase) or in twos or threes *i.e.* crowded (to simulate the conditions of the swarming phase).

As stated above it is generally believed that the development of the yellow pigment is connected with the maturation of the gonads. According to Vosseler [1905] ' the appearance of the yellow tint in the African Locust must be connected with the dissolution of the fat body, the yellow pigment being a bye-product that remains after the reserve material from the fat-body is used for building up the genital products [Uvarov, 1928].' Bücher, La Baume and others [1918], however, are of opinion that ' there is no direct physiological connection between the colour changes and the development of the genital products'. Their main argument is, that whereas the yellowing takes place simultaneously in both sexes, the female matures later than the male. They regard that the change in colour arises from senility rather than the development of the sex-glands. Our experiments have clearly shown that the development of yellow colour can take place even in the absence of the sex-glands.

Attention may also be drawn to another fact. Even the hoppers of *Schistocerca gregaria* in the gregarious phase may have intense yellow as the general colour of their body, black pigment in the cuticle forming a pattern over it. At the same time it has to be accepted that generally, if not always, the adults of the gregarious phase do develop yellow colour when sexually mature.

SEXUAL BEHAVIOUR OF DESEXUALISED ADULTS

The adults of which the gonads had been removed acted as normal individuals in their sexual behaviour. Indeed, they displayed marked tendency for copulation, which continued throughout the breeding period *i.e.* from the middle of January to the end of April. In one instance a female, without ovaries, was first provided with a normal male with which she was observed to copulate ten times. Subsequently she was provided with a male which had been castrated and they were observed to copulate fifty-five times. This female lived for 175 days and her castrated mate for 208 days. Similarly in another case where the ovary-less female had survived for 212 days, she was observed to copulate with a castrated male sixteen times. The duration of coitus ordinarily lasted from thirty minutes to five hours.

It is thus evident that the removal of the sex-glands does not inhibit the copulation reflex, on the other hand, the act which remains unconsummated is repeated oftener and is prolonged in duration, showing that sexual apetite is greatly intensified.

It has been observed that generally the females which have not copulated do not oviposit, but occasionally such females do lay eggs, which develop parthenogenetically. Parthenogenesis is by no means rare among locusts. It is a sigficant fact that the normal females mating with castrated males oviposited as frequently as the normal females mating with normal males, but when compared with the normal females that had never mated, normal females matured when the desexualised females laid a larger number of egg packets. This shows that while the sexual act, perhaps, provides the necessary stimulus for the processes connected with oviposition, the actual transference of sperms from the male to the female is not necessary to set up the reflex.

SUMMARY

Desexualised adults can live as long as the normal adults, and develop yellow pigment to the same degree as the normal insects. Their sexual behaviour remains unaltered. Thus for the development of the yellow coloration the presence of sex-glands is not necessary.

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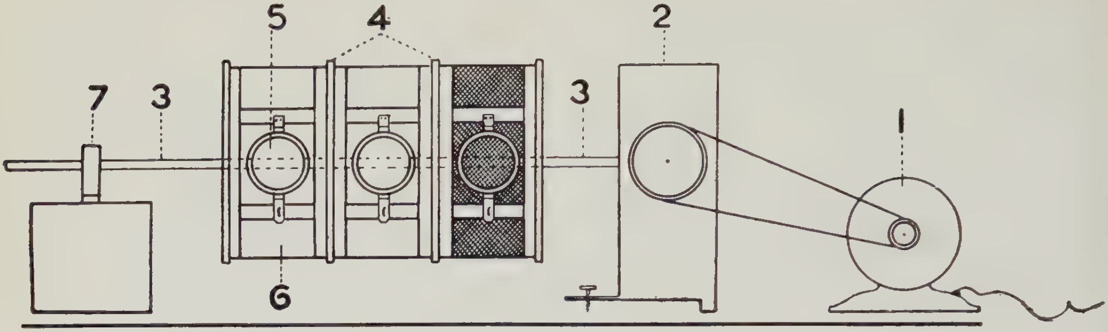

FIG. 1. REVOLVING WIRE GAUZE DRUMS :—1. electric motor ; 2. gear box ; 3. horizontal bar carrying the drums ; 4. glass sides of the drums ; 5. entrance to the drums ; 6. wire gauze side of the drum ; 7. ball-bearing support for the horizontal bar at its free end.

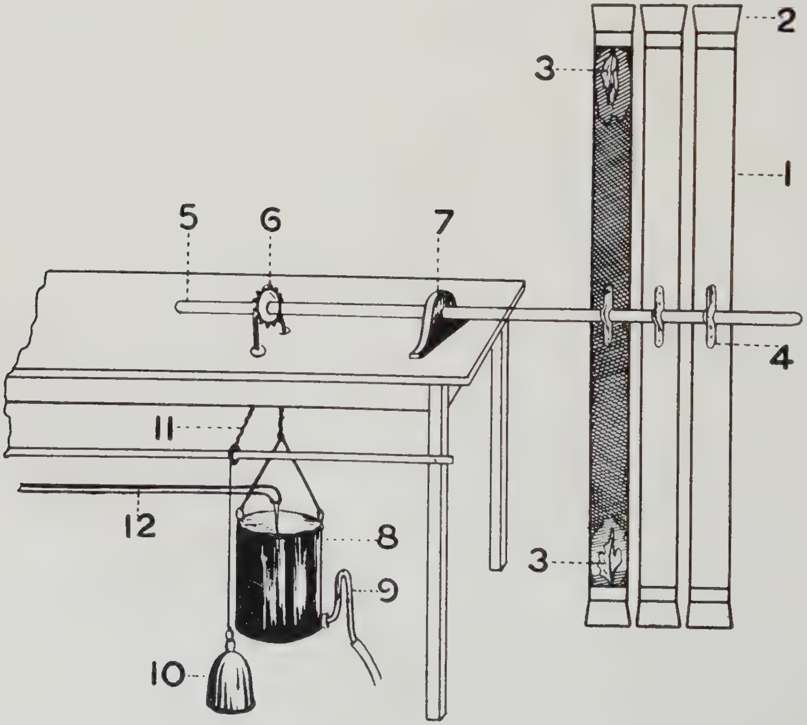

FIG. 2. SOMERSAULT CYLINDRICAL CAGES :—1. wire gauze cylindrical cage ; 2. cork stopper ; 3. leaf pinned for feeding ; 4. clip holding the cage to the horizontal bar, 5. ; 6. free-wheel ; 7. ball-bearing support ; 8. bucket with siphon arrangement, 9, at the bottom ; 10. the 'weight'; 11. bicycle chain attached to the weight and the bucket ; 12. water pipe running to the bucket.

STUDIES ON *SCHISTOCERCA GREGARIA* FORSK.

* V. PIGMENTATION AND PHYSICAL EXERTION

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(Received for publication on 6th January 1936)

(With Plates XXXIII-XXXV)

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I. Introduction [†]

The most characteristic feature by which the hoppers of the gregarious phase and those of the solitary phase stand out from each other is their coloration.

[†] Studies on *Schistocerca gregaria* Forsk.

I. The Micropyle in *Schistocerca gregaria* Forsk, and some other Acridiidae. *Ind. J. Agric. Sci.* **3** : 639-645.

II. The Biology of the Desert Locust with special relation to Temperature. *Ind. J. Agric. Sci.* **6** : 188-262.

III. Why do Locusts Eat Wool ? A Study in the Hydromania of *Schistocerca gregaria*. *Ind. J. Agric. Sci.* **6** : 263-67.

IV. Colour Changes and Sexual Behaviour in Desexualised *Schistocerca gregaria* Adults. *Ind. J. Agric. Sci.* **6** :586-90.

[†] This contribution is one of the series based on the work done under the Locust Research Scheme of the Imperial Council of Agricultural Research, India.

A '*gregarious*' or a swarming phase hopper, or a hopper bred crowded, is distinguished by the presence of a black pattern, which is absent in a '*solitary*' or a non-swarming phase hopper, or a hopper, bred in isolation the latter being a shade of green in colour. It is well known that one can change at will the coloration of a hopper born as a non-swarming phase hopper into the coloration typical of the swarming phase hopper, by breeding it under crowded conditions. And, conversely, one can change the coloration of a swarming phase hopper into the coloration of a typically non-swarming phase hopper by breeding it in isolation.

In nature the hoppers of the swarming phase live a very active life. They spend a major portion of the day, and under certain conditions even a portion of the night*, actively marching in band formations. Even during the periods of 'halt' they are in a state of agitation on account of excessive crowding. On the other hand, a non-swarming phase hopper spends its life in retirement in bushes and moves about only a little.

It appears that the presence of black pigmentation on the various parts of the body of a hopper and its active mode of living go hand in hand, while the absence of the black pattern is associated with an inactive mode of life. One depends upon the other and one leads to the other. For instance, the development and retention of the black pigment depends upon the activity of the individual, and it is the black pigmented hoppers which are more active. In the case of a non-swarming hopper, that causal factor—active life—being absent, the black pattern fails to appear. If that be the case, then a non-swarming hopper or a hopper bred isolated, if forced into activity, would, in spite of its solitary life, acquire the black pattern which is normally associated with a crowded life.

We arranged to force hoppers into this activity in two different ways. In one case the isolated hoppers of the non-swarming type were made to march a certain distance every day in specially devised apparatus. The second method simply consisted in keeping the isolated † non-swarming hoppers constantly in association with the hoppers or adults of some common grasshoppers and other Orthoptera.

The coloration of the hoppers from stage to stage in the experiments given in this paper has not been described in detail. Instead, to express the extent and intensity of black pigmentation, we have used certain terms. Typical cases are illustrated by coloured drawings. The terms employed are: '*Green type*', '*Intermediate*' and '*Black type*'.

'*Green type*' indicates a hopper which is completely free from black pigmentation and has green body colour. The five stages of green type hoppers are illustrated by Figs. 1 to 5 (Plate XXXIV). On the other hand a hopper having black pigmentation typical of the hoppers in a swarm, or in a crowd

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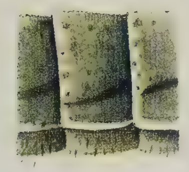
* Swarms of locust hoppers are known to continue their diurnal marches during hot and moonlit nights.

† Isolated from members of its own species.

TYPICAL GREEN HOPPERS



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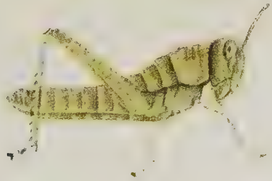
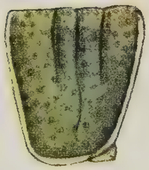


Fig. 1.



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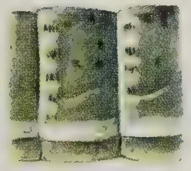

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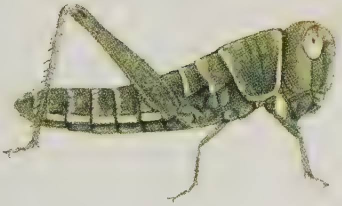
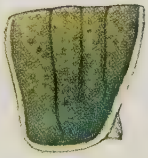


Fig. 2.



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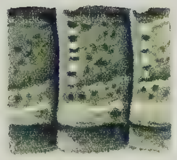

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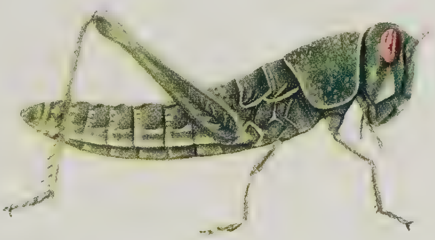
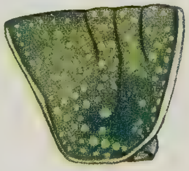


Fig. 3.



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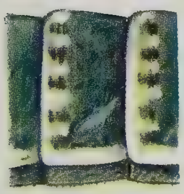

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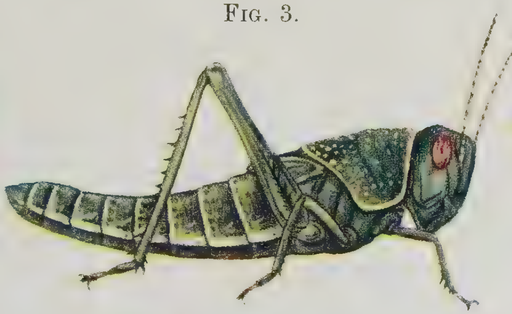
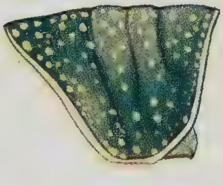


Fig. 4.



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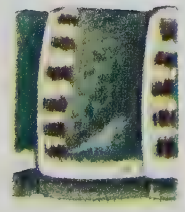

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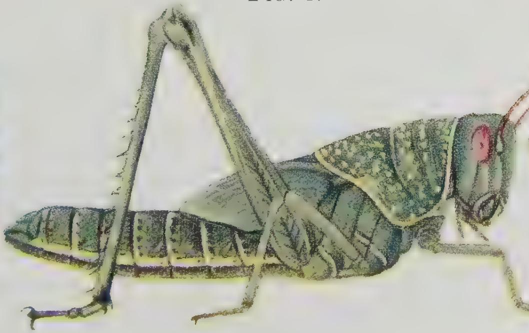
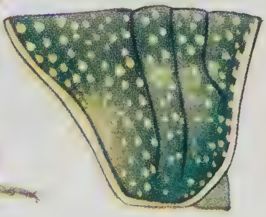


Fig. 5.



5*a*

Fig. 1. I stage (×2·5); Fig. 2. II stage (×2·5); Fig. 3. III stage (×1·5); Fig. 4. IV stage (×1·5); and Fig. 5. V stage (×1·1); with enlarged side views of the pronotum and some abdominal segments.

TYPICAL BLACK HOPPERS

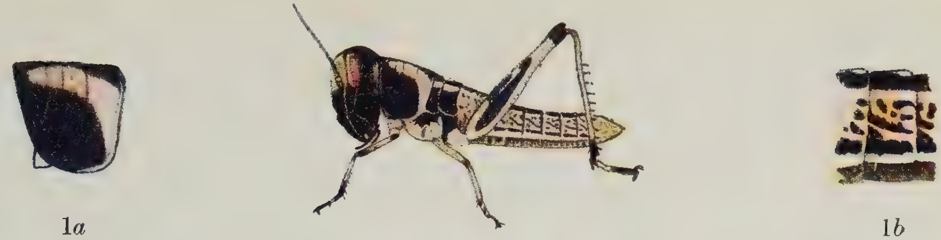

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FIG. 1.

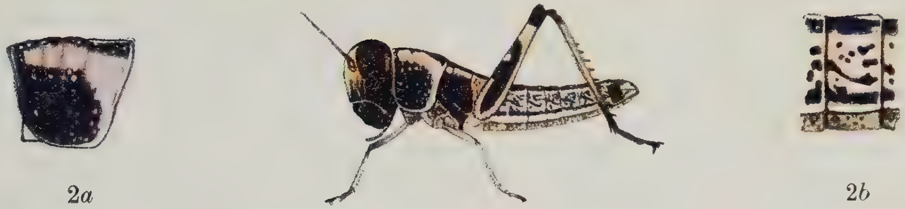
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FIG. 2.

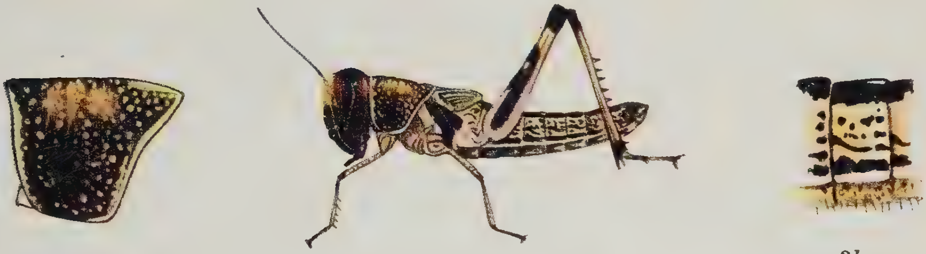
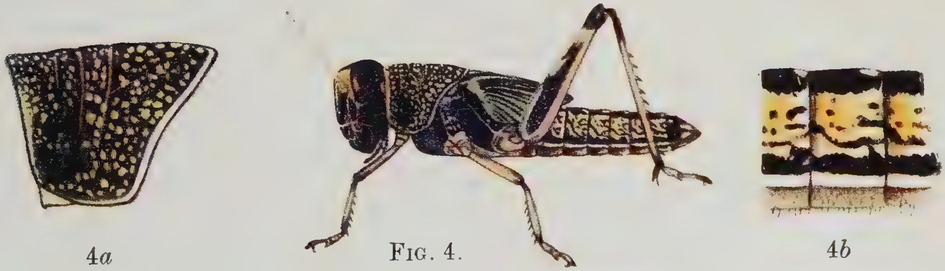
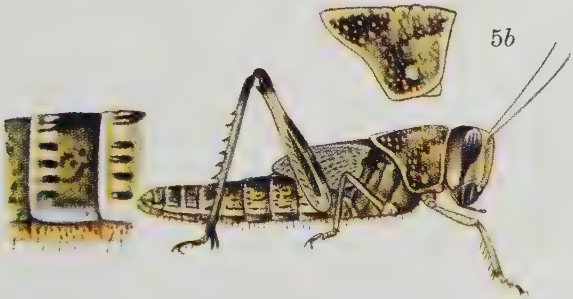

3a 3b

FIG. 3.


4a FIG. 4. 4b



5a FIG. 5.

GS. 1—4. Typical black hoppers: FIG. 1. II stage (×2·2); FIG. 2. III. stage (×1·3); FIG. 3. IV stage (×1·1); and FIG. 4. V stage (×0·8); with enlarged side views of the pronotum and some abdominal segments.

G. 5. An Intermediate hopper of the V stage (×0·8) with detailed side views of the pronotum and some abdominal segments.

(in the optimum temperature of about 30°C.) has been called '*Black type*'. *Black type* hoppers of 2nd to 5th stages are illustrated by figs. 1 to 4 (Plate XXXV). The term '*Intermediate*' indicates that state of black pigmentation which is midway between the '*Black type*' and the '*Green type*'. Thus a typical fifth stage '*Intermediate*' hopper appears like fig. 5 (Plate XXXV). Since all shades of black pigmentation between the '*Black type*' and the '*Green type*' appear, it has been found necessary to make further differentiations. Hoppers having more of black than the *Intermediate* have been described as '*Intermediate nearer black type*', while those having less of black have been described as "*Intermediate nearer green type*".

We have great pleasure in acknowledging the help of Mr. Munawwaruddin Siddiqi, Artist of the Entomological Section, Lyallpur, who prepared the plates for us.

II. Previous work

So far as we have been able to ascertain, only two investigators have attempted to test the influence of physical exertion on the development of black pigmentation in locust hoppers. Plotnikov [1927] worked on *Locusta migratoria* (Migratory Locust) and Faure [1932] on *Locustana pardalina* Walker (the Brown Locust).

Faure [1932] tried to force the hoppers of the Brown Locust into activity by a number of contrivances, of which his "Jarring apparatus" yielded some results. Of the 342 isolated hoppers that he subjected to "muscular exercise" only twenty-six (7·6 per cent) survived to the fifth stage. Of these only thirteen hoppers (15 per cent) maintained *solitaria* coloration, 11 hoppers (42 per cent) turned into *transience* and only 2 hoppers (7·7 per cent of the hoppers that reached V stage and 0·58 per cent of the total number of hoppers) acquired the '*gregaria*' coloration.

His experiments in which he crowded the hoppers of the Brown Locust with those of the other species are open to criticism. He placed a number of hoppers (six or seven) of *Locustana pardalina* in each cage in association with other hoppers. Six or seven hoppers of a species in a cage of the size he used (18 in. × 9 in. × 15 in.) would by themselves constitute a 'crowd' dense enough for the development of the characters of the phase, '*gregaria*'.

Although Plotnikov's experiments [1927] had yielded negative result, yet a brief review of his work will not be out of place.

To determine whether the excretory products were responsible for the development of the swarming coloration, Plotnikov, following Uvarov's advice, fed solitary hoppers of *Locusta migratoria* and *L. danica* on vegetation abundantly moistened with water extract of larval excreta. The result was negative.

To test if mechanical stimulation, caused by reciprocal jostling in a crowd, was instrumental in the production of the black pigment, he forced hoppers, which were reared isolated, to move for about two hours in glass globes fixed on a revolving wheel. However, 'the difficulty in judging the exact moment of moulting, when the hopper should be in a state of rest, as well as technical difficulties ', did not permit him 'to attain that state of mechanical irritation in the experiment which is received by a hopper moving in a swarm '. The result of this experiment also was negative.

In the following year (1926) he caused mechanical irritation to be applied for several minutes daily to solitary hoppers, in those regions in which the black patterns are present in crowded hoppers, by means of soft silk cord-like brushes fixed on a rapidly revolving small wheel. The moulting time was avoided. He carried on the experiment up to the fourth stage when the experiment was discontinued, because he saw no hope of obtaining the result he expected. He concludes : "Thus my idea as to the swarming coloration being caused by mechanical irritation of the skin must be given up ".

He sought for an explanation for the production of black pigment in starvation, cannibalism, temperature and humidity, but from his experiments he concluded that none of these factors caused the deposition of black pigment.

III. Physical exercise through muscular exertion

The construction of our apparatus, designed to force activity into the life of the hoppers bred in strict isolation, was based on the following characteristic behaviour of the hoppers of the Desert Locust.

It is a matter of common observation that these hoppers when placed in a breeding cage mostly remain near the top of the cage and towards the source of light. They also show a tendency to keep their heads pointed away from the ground. Bodenheimer [1930] has also described this behaviour. Taking advantage of these tendencies we used revolving wire gauze drums and somersault cylindrical cages, described below, to force hoppers into muscular exercise.

(A) REVOLVING WIRE-GAUZE DRUMS

The "revolving wire-gauze drums" (Plate XXXIII, fig. 1) consisted of six wire-gauze cylinders, four inches in diameter and three inches long. The two flat sides of the cylinders were of glass sheets *, each with a hole in the centre. Through these holes the cages were fixed on a horizontal bar, which was slowly revolved by means of an electric motor. The wire-gauze side was provided with a small circular entrance and door. The apparatus was so placed in the room and the rotation was so adjusted that the drum revolved from above downwards towards the brighter side of the room.

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* At first cardboard sides were provided for the drums. These forming a convenient parking place for the hoppers when the apparatus was worked, told upon the efficiency of the apparatus and had to be replaced by glass sheets.

A hopper placed in a cage of this type behaved in the following manner :—When the apparatus was not working the hopper approached the brighter side and climbed up the side of the drum to keep its head upwards. As the apparatus was worked the drum moved from above downwards. The hopper was thus carried down and brought in a darker place and in a horizontal position. To bring itself to the original position, the hopper marched upwards. But this attempt was constantly nullified by the slow and continuous movement of the drums with the result that the hopper continued to move on and on. Unfortunately all the hoppers, particularly in earlier stages, did not respond with the same readiness. It was, therefore, found necessary to introduce small pieces of pith or cork in each drum to act as 'whips' for the sluggish ones. The speed of revolution of the drums was adjusted to avoid over-carriage of the hoppers.

The apparatus was worked only during the day, between ten and seventeen hours, with an interval between thirteen and fourteen hours for rest and feeding. A drum revolved about fifty times an hour and its circumference was about 12·5 inches. Each hopper therefore could have a maximum walk of about 312 feet a day. The average distance walked was, however, shorter than this.

During the period that the drums were not revolving, the hoppers were either supplied with cotton or cabbage leaves placed in the drums, or they were transferred to these plants in pots. They were, however, kept in strict isolation. (Details are given under each experiment.)

(B) SOMERSAULT CYLINDRICAL CAGES

The use of bits of pith or cork, in revolving drums, however, brought in another factor, namely, the stimulus of contact. In order to avoid this we devised the following apparatus which worked more satisfactorily and gave more convincing results than the "revolving drums".

In this case also advantage was taken of the positively phototropic and negatively geotropic responses of the Desert Locust hoppers. The apparatus (Plate XXXIII, Fig. 2) consisted of wire-gauze cylindrical cages (3 feet long and 2 inches in diameter) arranged vertically and fixed to a horizontal bar along their middle. The horizontal bar was attached to a bicycle free-wheel over which ran a bicycle chain. One end of the chain was fixed to a bucket. This bucket had a siphon arrangement and when the level of water in the bucket reached the siphon level, the siphon began to work and emptied the bucket very quickly. To the other end of the chain was fixed a 'weight' which was heavier than the empty bucket, but lighter than the bucket with water at the siphon level. The bucket was placed under a tap and water dripped into it. When the water in the bucket reached the siphon level, the bucket became heavier than the 'weight' and went down, pulling the chain and thereby turning the horizontal bar round and rotating the cylindrical cages, through 180°. Soon after, the siphon started work, the bucket was emptied and the 'weight' went down pulling the bucket up. This movement, however, did not effect the horizontal rod and the cylindrical cages on account of the free-wheel arrangement. The cylindrical cages thus turned one way only, namely, with every downward movement of the bucket.

The open ends of these cylindrical cages were fitted with cork-stoppers, to each of which was pinned a green leaf, which served as food. The apparatus was set up in a dark room and near the top-ends of the cages was placed a 100 candle power electric lamp. The timing for the rotation of the cages was controlled by the speed with which the water dripped. After every twenty minutes or so the cages turned over.

The apparatus had several advantages over the "revolving drums". In the first place there was no foreign matter in the cages, and so the stimulus of contact was altogether eliminated. Secondly there was a provision for a constant supply of food inside each cage. The third very important advantage was that the interval between two turnings, even if it happened to be shorter than the period during which a hopper was able to traverse the whole length of the cylindrical cage, did not adversely affect the normal activities of the individual ; because : (*i*) the hopper, irrespective of its position in the cage, took a turn for the upper and more lighted end of the cage whenever the cage took the somersault ; and (*ii*) it was in a position to take rest whenever required and could moult without anything striking against its body. The last is particularly important, because an interference at the critical stage of moulting invariably results in death and it is this fact which accounts for the early deaths in a large number of cases in the 'revolving drums'. Lastly the apparatus could be worked continuously without constant attention and the hoppers did the maximum walking they were capable of doing.

(C) Observations

Experiment 1 (*Revolving drums*)

The hoppers in this experiment were taken from two hatchings.

Previous history.—The '*solitaria*' adults of both the pairs (P54 and P57) were collected at Pasni (Mekran coast) on 8th August 1932, and were received at Lyallpur on 19th August 1932. They were reared in pairs in wire-gauze cages (9 in. × 9 in. × 9 in.).

Female P54 laid eggs on 31st August 1932, which hatched on 14th September 1932. The hatching consisted of sixty-two green hoppers.

Female P57 laid eggs on 31st August 1932, which hatched on 14th September 1932. The hatching consisted of sixty-four green hoppers.

Thus all the hoppers had distinct '*solitaria*' tendency.

Conditions.—The experiment was started on 14th September 1932, and was conducted in the rearing room, the temperature during the experiment being :—

Highest maximum—35°C.
Lowest maximum—29°C.
Average maximum—31·08°C.

Highest minimum—32°C.
Lowest minimum—24°C.
Average minimum—27·13°C.

The food supplied was cotton leaves.

There was no pith or cork in the drums.

Experiment 2 (Revolving drums)

Previous history.—Reference No. of parents, P91.

The parents were collected at Sonmiani (Mekran Coast) and received at Lyallpur on 31st October 1932. They had marked "*solitaria*" characters and were reared in pairs.

The time of egg laying was not recorded. The hoppers hatched out on 1st February 1933—an all green hatching. Thus the hoppers had a distinct '*solitaria*' tendency.

Conditions.—The experiment was started on 1st February 1933 and was conducted in the hot house which was artificially heated. The temperature during the period was :—

Highest maximum—37·2°C.
Lowest maximum—27·2°C.
Average maximum—32·05°C.

Highest minimum—30·3°C.
Lowest minimum—25·5°C.
Average minimum—27·5°C.

The hoppers were removed from the drums after exercise and placed on cabbage plant for feeding. Pith and cork pieces were also placed in the drums.

Experiment 3 (Revolving drums)

Previous history.—Reference No. of parents, P136.

The parents were of the '*solitaria*' type and were received from Pasni on 8th August 1933. They were reared as a pair in the laboratory. The eggs were laid on 2nd August 1933 and the hoppers hatched out on 15th August 1933, the hatching consisting of forty-five green hoppers. Thus the hoppers had marked '*solitaria*' tendency.

Conditions.—The experiment was started on 15th August 1933 and was conducted in the rearing room, the temperature during the experiment being :—

Highest maximum—37·7°C.	Highest minimum—31·0°C.
Lowest maximum—28·0°C.	Lowest minimum—26·6°C.
Average maximum—30·87°C.	Average minimum—28·0°C.

Cotton leaves were supplied as food.

Pith and cork pieces were also placed in the drums.

Experiment 4 (Somersault cages)

Previous history.—Reference No. of parents, P110.

The parents were collected from Sonmiani where they were found in solitary state, but on receipt at Lyallpur on 31st October 1932 were described as "not having typical '*solitaria*' characters". They were reared in pairs.

The eggs were laid on 6th July 1932 and the hoppers hatched out on 30th December 1932. The hatching was a mixed one and consisted of fifty-eight green and seventeen intermediate hoppers. Pure green hoppers were selected for this experiment. Four of them (P110/2, P110/3, P110/4 and P110/5) were reared isolated on cotton plants during the first stage and were placed in the experimental cages after the first moult. They were green in the first as well as in the second stage. The fifth hopper (P110/6) was reared isolated on cotton plant during the first, second and third instars, and from the beginning of the fourth instar onwards it was reared in a cylindrical cage of the "somersault apparatus". During all the instars, including the fourth this hopper was of green colour.

Thus, in all cases, before subjecting the hoppers to experimental conditions, their *solitaria* tendency was established.

Conditions.—The experiment was started on 7th January 1933 and was conducted in a dark room artificially heated by means of electric heaters. The temperature during the period was :—

Highest maximum—35°C.
Lowest maximum—30°C.
Average maximum—33°C.

Highest minimum—28°C.
Lowest minimum—24°C.
Average minimum—26·5°C.

Cotton leaves were provided for feeding and were pinned on to the corks of the cylindrical cages. The leaves were changed thrice a day, at 9, 13 and 18 hours.

(D) REMARKS

In Table I, has been described the extent of black pigmentation obtained by the hoppers from instar to instar in experiments (1) to (4). In experiment (1) the experimental hoppers did acquire black pigmentation, but not to the degree which was typical of the hoppers reared crowded at that temperature. Thus the blackest hopper of this experiment could only be described as '*Intermediate*'. In this experiment no pith or cork pieces were introduced into the cages and in the absence of this material, which acted as a sort of 'whip', the hoppers did not undergo the amount of physical exertion necessary for the development of the degree of black pigmentation typical of a '*Black type*' hopper at this temperature. We give the duration of the life cycle of these hoppers in Table II. The control hoppers remained green.

Pith and cork pieces were introduced in experiments (2) and (3) and black pigmentation obtained in these was more extensive and intensive than in experiment (1) (Table I). In fact, all the hoppers, in experiment (2) were described as '*Black Type*' in the last hopper stage, and all the hoppers in experiment (3) had acquired the degree of black pigmentation which was indeed very near the '*Black type*'. All the control hoppers were, of course, of the '*Green type*' throughout. Tables III and IV give the duration of life-cycle of the hoppers in experiments (2) and (3).

The fact that all the hoppers in experiment (4) (Somersault cages) acquired in the fifth stage the black pigmentation of the intensity of the '*Black type*' hopper is particularly significant, in view of the fact that one of the hoppers (P110/6) was forced into activity during one stage only, namely, the fourth stage, and the other four hoppers during three of the hopper stages. It may be pointed out once again that while these hoppers in experiment (4) were forced to march to their maximum capacity, the somersault apparatus working continuously, the hoppers in experiments (1), (2) and (3) were forced into activity for about six hours a day only. This explains the quicker appearance of the black pigmentation in experiment (4). The duration of the hopper stages of this experiment is given in Table V.

TABLE I

Extent of pigmentation from instar to instar of the isolated hoppers forced into activity

Experiment No.	Condition	Reference No. of hoppers	I stage	II stage	III stage	IV stage	V stage	Remarks
1	Revolving drums (without pith or cork).	P54-57/E 1	Green type	Intermediate nearer green type.	Intermediate	Intermediate (died).	...	
		P54-57/E 2	Do.	Intermediate	Intermediate nearer green type (died).	...	...	
		P54-57/E 3	Do.	Do.	Do.	...	...	
		P54-57/E 4	Do.	Intermediate nearer green type.	Do.	Intermediate nearer green type (died).	...	
	Control	P54-57/Cont. 1.	Do.	Green type	Green type	Green type	Green type	
		P54-57/Cont. 2.	Do.	Do.	Intermediate nearer green type.	Do.	Do.	
		P54-57/Cont. 4.	Do.	Do.	Green type	Do.	Do.	
2	Revolving drums (with pith and cork).	P91/R 1	Do.	Almost black type.	Black type	Black type	...	
		P91/R 2	Do.	Intermedeiate	Black type (Escaped).	...	...	
		P91/R 3	Do.	Intermediate nearer green type.	Black type	Black type* (died).	...	* Hopper died during moult. The parts that were exposed showed that the hopper was black type.
		P91/R 4	Do.	Do.	(*a*) Black type (*b*) Black type (died).	...	...	(*b*) Extra moult.
	Control	P91/R 5	Do.	Almost green	Green type	Green type	...	
		P91/R 6	Do.	Do.	Do.	Do.	...	

Experiment No.	Condition	Reference No. of hoppers	I Stage	II Stage	III Stage	IV Stage	V Stage	Remarks
3	Revolving drums (with pith and cork).	P136/15.8.33 A	Do.	Intermediate nearer green type.	Intermediate nearer green type.	Intermediate nearer black type.	Intermediate nearer black type.	
		P136/15.8.33 B	Do.	Intermediate nearer black type.	Intermediate	Do.	...	Escaped during the IV stage.
		P136/15.8.33 C	Do.	Intermediate nearer green type.	Intermediate nearer green type.	Intermediate	Intermediate nearer black type.	
		P136/15.8.33 E	Do.	Do.	Do.	Do.	Do.	
	Control	P136/15.8.33 1.	Do.	...	...	Green type	Green type.	
4	Somersault cages	P110/2	Green type.	Green type	*	Intermediate	Intermediate nearer black type.	During I instar the hoppers were reared isolated on plants. After the 1st moult they were transferred into experimental cages. * The colouration in the third instar was not described.
		P110/3	Do.	Do.	*	Black type	Black type.	
		P110/4	Do.	Do.	*	Do.	Do.	
		P110/5	Do.	Do.	*	Intermediate nearer black type.	Do.	
		P110/6	Do.	Do.	Green type	Green type	Almost black type.	This hopper was kept isolated on the plant during I, II and III instars. At the beginning of the fourth instar when it was transferred to experimental cage it was a pure green hopper.

TABLE II

Life history of hoppers in experiment 1

Reference :—No. P54-57.

Temperature :—Average maximum—31·08°C.
Average minimum—27·13°C.

Date	Experimental				Control		
	E 1	E 2	E 3	E 4	Cont. 1	Cont. 2	Cont. 4
14th Sept. 1932 .	I	I	I	I	I	I	I
15th ,, ,, .							
16th ,, ,, .							
17th ,, ,, .							
18th ,, ,, .							
19th ,, ,, .	II	II			II	II	II
20th ,, ,, .				II			
21st ,, ,, .							
22nd ,, ,, .			II				
23rd ,, ,, .							
24th ,, ,, .							
25th ,, ,, .					III		
26th ,, ,, .						III	III
27th ,, ,, .							
28th ,, ,, .	III						
29th ,, ,, .							
30th ,, ,, .			III	III			
1st Oct. ,, .		III					
2nd ,, ,, .		(D)					
3rd ,, ,, .							
4th ,, ,, .							

NOTE.—D indicates death of the hopper.

TABLE—II—*contd*

Dates	Experimental				Control		
	E 1	E 2	E 3	E 4	Cont. 1	Cont. 2	Cont. 4
5th Oct. 1932 .			(D)				
6th „ „ .							
7th „ „ .					IV		
8th „ „ .							IV
9th „ „ .						IV	
10th „ „ .							
11th „ „ .							
12th „ „ .	IV						
13th „ „ .				IV (D)			
14th „ „ .							
15th „ „ .	(D)				V	V	
16th „ „ .							V

NOTE.—D indicates death of hopper.

TABLE III

Life history of hoppers in experiment 2

Reference :—No. P91
Temperature of hot house :—Average Maximum—32·05°C.
Average Minimum—27·5°C.

Dates	Experimental				Control	
	R1	R2	R3	R4	R5	R6
1st Feb. 1933 . . .	I	I	I	I	I	I
2nd „ „ . . .						
3rd „ „ . . .						
4th „ „ . . .						
5th „ „ . . .						

TABLE—III—*contd*

Dates	Experimental				Control	
	R 1	R 2	R 3	R 4	R 5	R 6
6th Feb. 1933	II				II	II
7th ,, ,,			II			
8th ,, ,,		II		II		
9th ,, ,,						
10th,, ,,						
11th,, ,,						
12th,, ,,						
13th,, ,,						
14th,, ,,					III	III
15th,, ,,						
16th,, ,,	III		III	III (*a*)		
17th,, ,,		III				
18th,, ,,						
19th,, ,,		Escaped.				
20th,, ,,				III (*b*)		IV
21st ,, ,,						
22nd,, ,,					IV	
23rd,, ,,						
24th,, ,	IV					
25th,, ,,				(D)		
26th,, ,,						
27th,, ,,	(D)		IV			
28th,, ,,			(D)			

NOTE.—D indicates death of the hopper.
III (*a*) and III (*b*) represent the normal and the extra third stage.

TABLE IV

Life history of hoppers in experiment 3

Reference :—No. P136

Temperature of rearing room:— Average maximum—30·87°C.
Average minimum—28·0°C.

Dates	Experimental				Control
	A	C	E	B	I
15th Aug. 1933	I	I	I	I	I
16th ,, ,,					
17th ,, ,,					
18th ,, ,,					
19th ,, ,,					
20 h ,, ,,					
21st ,, ,,					
22nd ,, ,,					
23rd ,, ,,					
24th ,, ,,	II		II		
25th ,, ,,					
26th ,, ,,				II	
27th ,, ,,					
28th ,, ,,					
29th ,, ,,					
30th ,, ,,					
31st ,, ,,		II			
1st Sept. 1933					
2nd ,, ,,					

TABLE IV—*contd.*

Dates	Experimental				Control
	A	C	E	B	I
3rd Sept. 1933 . . .					
4th ,, ,, . . .					
5th ,, ,, . . .					
6th ,, ,, . . .					
7th ,, ,, . . .					
8th ,, ,, . . .					
9th ,, ,, . . .					
10th ,, ,, . .					
11th ,, ,, . .					
12th ,, , . . .					IV
13th ,, ,, . . .					
14th ,, , . . .			IV	IV	
15th ,, ,, . . .					
16th ,, ,, . . .	IV	IV			
17th ,, ,, . . .					IV
18th ,, ,, . . .					
19th ,, ,, . . .					
20th ,, ,, . . .					A
21st ,, ,, . . .					
22nd ,, ,, . . .					
23rd ,, ,, . . .					
24th ,, ,, . . .			V		
25th ,, ,, . . .	V	V			
26th ,, ,, . . .					

TABLE V

Life history of hoppers in experiment 4

Reference :— No. P110.

Temperature of dark room :—Average maximum—33°C.
Average minimum—26·5°C.

Dates	Experimental				
	2	3	4	5	6
30th Dec. 1932 . .	I	I	I	I	I
31st „ „ . . .					
1st Jan. 1933 . . .					
2nd „ „ . . .					
3rd „ „ . . .					
4th „ „ . . .					
5th „ „ . . .					
6th „ „ . . .					
7th „ „ . . .	II	II	II	II	
8th „ „ . . .					
9th „ „ . . .					
10th „ „ . . .					
11th „ „ . . .					
12th „ „ . . .					
13th „ „ . . .					
14th „ „ . . .					
15th „ „ . . .					
16th „ „ . . .	IV	IV	IV	IV	
17th „ „ . . .					IV
18th „ „ . . .					

TABLE V—*contd.*

Dates	Experimental				
	2	3	4	5	6
19th Jan. 1933 . .					
20th „ „ . . .					
21st „ „ . . .					
22nd „ „ . . .					
23rd „ „ . . .	V	V	V	V	
24th „ „ . . .					
25th „ „ . . .					
26th „ „ . . .					V

IV. Physical exercise through association with grasshoppers of other species

Faure confined *Locustana pardalina* hoppers with those of *Locusta migratoria migratorioides*, and *Locusta migratoria* hoppers with the hoppers of *Schistocerca gregaria* or *L. pardalina*. In all the five experiments that he performed, he kept more than one hopper of the species under experiment in each cage. This makes the results inconclusive. The size of the cage, the number of experimental hoppers reared in each cage and the number of associate hoppers in Faure's experiments were as follows :—

Serial No.	Experimental hoppers		Size of cage	Associates		Results
	Name	No.		Name	No.	
1	*L. pardalina* .	7	18in. × 9in. × 15in. .	*L. m. migratorioides.*	400	Four reached V stage; all good gregaria type.
2	Ditto .	6	Ditto .	Ditto .	400	One survived to IV stage and was described as good gregaria.
3	*L. m. migratorioides.*	5	Ditto .	*Schistocerca gregaria.*	250	Two reached V stage; good transiens near gregaria type.
4	Ditto .	6	6in. × 4·5in. × 6in. .	*L. pardalina* .	65	One survived to IV stage; Gregaria type.
5	Ditto .	3	Ditto .	Ditto .	50	Ditto.

When more than one hopper of a species are confined in a small cage, as Faure did, it cannot be claimed that the '*gregaria*' characters obtained were not the result of intra-specific crowding.

In all the five experiments performed by us in each cage only one '*solitaria*' hopper of the Desert Locust was in association with a number of other Orthoptera. In three of these experiments the associate insects were the hoppers of *Poecilocerus pictus*; in the fourth experiment a miscellaneous collection of grasshoppers (predominatingly *Chrotogonus* sp.) was associated. In the fifth experiment the associates were the adults of the house cricket (*Gryllus domesticus*). The last experiment is particularly interesting inasmuch as the association did not result in the production of the black pigmentation in the *solitaria* hoppers of the Desert Locust.

Experiment 5

Previous history.—Reference No. of the parents, K22.

The parents were found in solitary state at Angaria Gadap (near Karachi). They were collected on 30th February 1932 and were received at Lyallpur on 4th March 1932. They were bred as a pair, in a cage 9 in. × 9 in. × 9 in.

The eggs were laid on 26th April 1932 and hatched on 10th May 1932. The hatching was a mixed one. Only the '*solitaria*' hoppers were employed in this experiment. Thus the hoppers used were '*solitaria*' even though they belonged to a mixed hatching. The hoppers may be described as having a moderate '*solitaria*' tendency.

Associates.—The associates were the hoppers of *P. pictus*. At the start of the experiment three second instar hoppers of *P. pictus* were introduced in each cage. This strength was maintained throughout the experiment, the dead and the disabled ones being replaced from time to time with fresh hoppers from the field.

Conditions.—The cage was a wire—gauze cylinder six inches in height and five inches in diameter. It was fixed on a cabbage plant, the latter serving as food material. The experiment was conducted at room temperature which during the period was :—

Highest maximum—38·5°C.	Highest minimum—32°C.
Lowest maximum—33°C.	Lowest minimum—26°C.
Average maximum—35·7°C.	Average minimum—29·7°C.

Remarks.—From the record of the control isolated hoppers (K22/10.V.32, 1, 2, 3, 4, 5 and 7) it is clear that the *solitaria* hoppers of this batch, when bred isolated, maintained their '*solitaria*' coloration. This established the '*solitaria*' tendency of the hoppers of this experiment (Table VI). The hoppers that were associated with three *P. pictus* hoppers assumed gregaria coloration without exception. We can conclude, that in association with three *P. pictus* hoppers, in a space of the size of the cage employed in this experiment, a single hopper of *Schistocerca gregaria* assumes the coloration typical of a crowded hopper at this temperature. The progress of black pigmentation in these hoppers from instar to instar is given in Table VI. Table VII gives the life history of these hoppers.

TABLE VI

Progress of pigment formation in experiment 5

Reference No.	I stage	II stage	III stage	IV stage	V stage	Adult	Remarks
				Experimental			
K22/10-5-32 . 11	Pure green	Intermediate	Almost black type.	Almost black	Black type	Gregarious	One *Schistocerca gregaria* hopper with three *P. pictus*-hoppers.
K22/10-5-32 . 12	Do.	Black type	Black type	Black type	Black type	Do.	One *Schistocerca gregaria* hopper with three *P. pictus* hoppers.
K22/10-5-32 . 13	Do.	Black type	Black type	Almost black type.	Black type	Do.	Ditto.
				Control isolated			
K22/10-5-32 . 1	Do.	Intermediate nearer green type.	Intermediate nearer green type.	Intermediate	Intermediate	Solitary	Control Isolated.
K22/10-5-32 . 2	Do.	Almost green type.	Green type	Green type	Green type	Do.	Do. Do.
K22/10-5-32 . 3	Do.	Almost green type.	Almost green type.	Green type	...	...	Do. Do.
K22/10-5-32 . 4	Do.	Intermediate	Almost green type.	Almost green type.	...	...	Do. Do.
K22/10-5-32 . 5	Do.	Green type	Green type	Green type	Green type	...	Do. Do.
K22/10-5-32 . 7	Do.	Almost green type.	Green type	Green type	Green type	Solitary	Do. Do.
				Control crowded			
K22/10-5-32 . 8	Do.	a. Black type b. *	a. Black type ...	a. Intermediate nearer black type. ...	a. Intermediate nearer black type. ...	a. Intermediate ...	Do. Two in a cage *Died during the 1st moult.
K22/10-5-32 . 9	Do.	a. Black type b. Black type	a. Black type b. Black type.	a. Black type b. Black type.	a. Black type b. Black type.	a. Gregarious b. Gregarious.	Control Two in a cage.
K22/10-5-32 . 10	Do.	a. Black type b. ... c. ...	a. Almost black	Intermediate	Intermediate	Intermediate	Control. Three in a cage-1 died on second day, 2nd died at 1st moult, 3rd carried on to VI stage.

TABLE VII

Life history of hoppers in experiment 5

Reference No. K 22/10. V. '32

Temperature:—Highest maximum—38·5°C. Highest minimum—32·6°C.
Lowest maximum—33·0°C. Lowest minimum—26·0°C.
Average maximum—35·7°C. Average minimum—29·7°C.

Date (1932)	Experimental			Control, isolated						Control, crowded		
	11	12	13	1	2	3	4	5	7	8	9	10
10th May .	I	I	I	I	I	I	I	I	I	I (2)	I (2)	I (3)
11th ,, .												
12th ,, .												
13th ,, .												
14th ,, .												
15th ,, .				II								
16th ,, .	II	II	II					II			II (2)	I (2) II (1)
17th ,, .					II	II	II		II	II I (D)		1 (2) (D) II 1
18th ,, .												
19th ,, .												
20th ,, .	III	III	III	III				III			III	
21st ,, .					III	III	III		III			III
22nd ,, .										III		
23rd ,, .								IV			IV (2)	
24th ,, .	IV			IV		IV				IV		
25th ,, .		IV	IV		IV		IV		IV			IV
26th ,, .												
27th ,, .							Died.	V			V (2)	
28th ,, .				V	V					V		
29th ,, .	V					IV (Died)			V			V
30th ,, .		V	V									
31st ,, .												
1st June .												
2nd ,, .												
3rd ,, .										A 1		
4th ,, .			A 1	A 1	A 1						A 2	A 1
5th ,, .		A 1										
6th ,, .	A											
7th ,, .									A 1			

Note.—Numbers in brackets indicate the thickness of crowd.
D indicates death of the hopper.

Experiment 6

Previous history.—Reference No. of parents, K14.

The parents belonged to the same field collection as those in experiment (5).

The date on which the eggs were laid was not recorded.

The hoppers hatched out on 13th May 1932. The hatching consisted of fifty-four green hoppers only. Thus the '*solitaria*' tendency in the hoppers employed in this experiment was more marked than in the hoppers employed in experiment (5).

Associates.—*P. pictus* hoppers were the associates. Their strength was maintained at three throughout the experiment.

Conditions.—The hoppers were reared in cylindrical wire-gauze cages six inches long and five inches in diameter. In the laboratory the temperature during the period was :—

Highest maximum—38·8°C.	Highest minimum—32°C.
Lowest maximum—32·4°C.	Lowest minimum—28°C.
Average maximum—34·03°C.	Average minimum—29·01°C.

Remarks.—The experiment is similar to the previous experiment with regard to :—(*a*) space, (*b*) number and species of the associates, and (*c*) temperature.

Table VIII gives the stage-to-stage coloration of the hoppers. It shows that while the isolated hoppers in control continued to be '*Green type*' throughout the hopper stages, those in association varied from '*Green type*' to '*Black type*'.

It would seem that for a hopper, that belongs to a pure green hatching and is born of *solitaria* parents, an association with three *P. pictus* hoppers, in the space provided in this experiment, creates that 'neutral' state which may either lead to the production of black pigmentation or may remain ineffective through such minor individual differences as, (*i*) the slightly bigger or smaller size of the food plant affecting the available space for the movements of the hoppers, (*ii*) the small individual variations in the activity of the associates, and (*iii*) the individual difference in the physiology of the hoppers themselves. In experiments on crowding one finds that the extent and intensity of pigmentation depends upon the intensity of population per unit area of space. The life cycle of hoppers is given in Table IX.

TABLE VIII

Extent of black pigmentation from instar to instar of the isolated hoppers in experiment 6

Reference No.	I stage	II stage	III stage	IV stage	V stage	Adult	Remarks
K14/11	Green type	Black type	Black type	Black type	Black type	Gregaria	One *Schistocerca gregaria* hopper associated with 3 *P. pictus* hoppers.
K14/12	Do.	Intermediate nearer black type.	Intermediate	Intermediate nearer green type.	Almost green type.	Transient	
K14/13	Do.	Do.	Do.	Green type	Green type	Solitaria	
K14/14	Do.	Intermediate	Do.	Intermediate	Do.	...	
K14/1	Do.	Green type	Green type	Green type	Green type	Solitaria	Control. Isolated hoppers.
K14/2	Do.	Almost green type.	Do.	Do.	Do.	Do.	
K14/3	Do.	Do.	Do.	Do.	...	...	
K14/5	Do.	Green type	Do.	Do.	Green type	Solitaria	
K14/7	Do.	(a) Black type (b) Black type	(a) Black type (b) Black type	(a) Black type (b) Black type	(a) Black type (b) Black type	(a) Gregaria (b) Gregaria	Control. Two hoppers of *Schistocerca gregaria* in a cage.
K14/8	Do.	(a) Black type (b) Black type	(a) Black type (b) Black type	(a) Black type (b) Black type	(a) Black type (b) Black type	(a) Gregaria (b) Gregaria	Ditto.
K14/9	Do.	(a) Black type (b) Black type (c) Black type	(a) Black type (b) Black type (c) Black type	(a) Black type (b) Black type (c) Black type	(a) Black type (b) Black type (c) Black type	(a) Gregaria (b) Gregaria (c) Gregaria	Control. Three hoppers of *Schistocerca gregaria* in a cage.

TABLE IX

Life-history of hoppers in experiment 6

Reference :—No. K14.

Temperature :—Highest maximum—38·8°C. Highest minimum—32°C.
Lowest maximum—32·4°C. Lowest minimum—28°C.
Average maximum—34·03°C. Average minimum—29·01°C.

Date (1932)	Experimental				Control, isolated				Control, crowded		
	11	12	13	14	1	2	3	5	7	8	9
13th May .	I	I	I	I	I	I	I	I	1 (2)	1 (2)	1 (3)
14th " .											
15th " .					II						
16th " .											
17th " .											
18th " .	II	II	II				II		I (1) II (1)	II (2)	II (3)
19th " .				II		II		II	II (2)		
20th " .											
21st " .											
22nd " .							III				
23rd " .	III	III	III	III	III	III		III	III (2)	III (2)	III (3)
24th " .											
25th " .	IV	IV	IV	IV			IV		IV (1) III (1)	IV (2)	III (1) IV (2)
26th " .					IV	IV		IV	IV (2)		IV (3)
27th " .							Died				
28th " .											
29th " .											
30th " .	V	V	V	V	V					V (2)	IV (2) V (1)
31st " .						V		V			V (3)
1st June .									V (2)		
2nd " .											
3rd " .											
4th " .											
5th " .			A								
6th " .		A		V (D)		A			A (1) V (1)	A (1) A (1)	A (2) V (1)
7th " .	A				A			A		A (2)	
8th " .									A (2)		A (3)

Note.—Numbers in brackets indicate the thickness of crowd.

Experiment 7

Previous history.—The hoppers for this experiment were taken from two hatchings.

Reference Nos. of the parents, K5 and K17.

The parents were of the phase '*solitaria*' and belonged to the same collection as those in experiments (1) and (2). They were bred in pairs. The eggs were laid on 30th April 1932 and hatched on 19th May 1932. The hatchings from K5 and K17 consisted of fifty-seven and fifty-one green hoppers respectively. All the hoppers therefore had a marked '*solitaria*' tendency.

Associates.—During its first stage each *Schistocerca gregaria* hopper had six hoppers of *P. pictus* as its associates. Subsequently, however, the number of *P. pictus* hoppers varied from stage to stage and from hopper to hopper. The number of associates in each case is indicated in Table X.

Conditions.—Each *Schistocerca gregaria* hopper in company with its associate hoppers was reared on a cabbage plant in a cylindrical wire-gauze cage six inches high and five inches in diameter. The experiment was conducted in the laboratory and the temperature during the period was :—

Highest maximum—40°C.	Highest minimum—33°C.
Lowest maximum—34°C.	Lowest minimum—28°C.
Average maximum—36·5°C.	Average minimum—30·05°C.

Remarks.—The experiment was similar to experiment (6) with regard to (*a*) space and (*b*) '*solitaria*' tendency of the hoppers. The temperature in this experiment was slightly higher than in the previous experiments and was thus a little less favourable for the acquisition of '*gregaria*' coloration. The number of associates was generally higher. All the hoppers in an association of such thickness have assumed the '*gregaria*' coloration. The state of black pigmentation from instar to instar is indicated in Table X and the life cycle of these hoppers is given in Table XI.

TABLE X

Progress of black pigmentation in experiment 7

Ref. No.	I stage	II stage	III stage.	IV stage	V stage	Adult	Remarks
(Single *Schistocerca gregaria* hopper crowded with *P. pictus* hoppers)							
K5-17/1	*Green type P. p.* 6	*Black type P. p.* 6	*Black type P. p.* 6	*Black type P. p.* 8	*Black type P. p.* 4	Gregarious	Male
K5-17/2	*Green type P. p.* 6	*Black type P. p.* 8	*Black type P. p.* 8	*Black type P.* p. 8	*Black type P. p.* 1	Almost gregaria	Female
K5-17/3	*Green type P. p.* 6	*Black type P. p.* 6	*Black type P. p.* 8	*Black type P. p.* 8	Intermediate very near *black type P. p.* 4	Gregaria	Female
K5-17/5	*Green type P. p.* 6	*Black type ?*	*Black type P. p.* 8	*Black type P. p.* 8	*Black type P. p.* 4	Almost gregaria	Male
[Isolated *Schistocerca gregaria* hopper (control)]							
K5-17/6	Green type	Green type	...	...	...	...	
K5-17/7	Green type	Green type	Green type	Green type	Green type	Solitaria	Female
[Crowded *Schistocerca gregaria* hoppers (control)]							
K5-17/8	*Green type S. g.* 4	*Black type S. g.* 4	*Black type S. g.* 4	*Black type S. g.* 3	*Black type S. g.* 3	Gregaria	

NOTE.—*P. p.*=*Peocilocerous pictus* hoppers.

S. g.—*Schistocerca gregaria* hoppers.

The number given below the colour of a stage show the strength of the crowd of the *P. pictus* hoppers or *Schistocerca gregaria* hopper, as specified, during stage. The effect of crowding appears in the next stage.

TABLE XI

Life—history of hoppers in experiment 7

Temperature :—Heighest maximum—40°C. Highest minimum—33°C.
Lowest maximum—34°C. Lowest minimum—28°C.
Average maximum—36·05°C. Average minimum—30·05°C.

Date (1932)	Experimental				Control		
	K5-17 1	K5-17 2	K5-17 3	K5-17 5	Cont. K5-17/6	Cont. K5-17/7	Cont. K5-17/8
20th May	I	I	I	I	I	I	I
21st ,,							
22nd ,,							
23rd ,,		II					
24th ,,	II		II	II		II	II (4)
25th ,,					II		
26th ,,							
27th ,,	III	III	III	III			III(3) II(1)
28th ,,						III	III(4)
29th ,,							
30th ,,				IV			III(3) III(D)
31st ,,	IV	IV	IV		Died II		IV(3)
1st June						IV	
2nd ,,							
3rd ,,							
4th ,,			V	V			IV(2) V(1)
5th ,,	V	V				V	
6th ,,							
7th ,,							
8th ,,							
9th ,,							
10th ,,				A			
11th ,,	A	A1	A1				
12th ,,						A1	A(3)

NOTE.—Numbers in brackets indicate the thickness of crowd.
D indicates death of hopper.

Experiment 8

Previous history.—The hoppers in this experiment were taken from two hatchings.

Reference No. of the parents, P 54 and P 57.

The parents were collected at Pasni on 8th August 1932 and received at Lyallpur on 17th August 1932. They were of '*Solitary*' phase and were reared in pairs.

The eggs were laid on 31st August 1932 and hatched on 14th September 1932. The hatchings of P54 and P57 consisted of sixty-three and sixty-four green hoppers respectively. The hoppers thus had a cent per cent '*solitaria*' tendency.

Associates.—Each *Schistocerca gregaria* hopper had fifteen adult common grasshoppers (predominatingly *Chrotogonus* sp.) as its associates. Fresh catches from the fields replaced the dead associates.

Conditions.—Each hopper of the Desert Locust along with its fifteen associates was reared in a cubical cage measuring 6 in. × 6 in. × 6 in. and with four sides of wire gauze, the botton of wood and the top of a sliding glass sheet. Fresh cotton leaves were dropped into the cage four times a day. The room temperature during the period was:—

Highest maximum—35·0°C. Highest minimum—32·0°C.
Lowest maximum—21·6°C. Lowest minimum—16·6°C.
Average maximum—26·08C. Average minimum—22·03°C.

Remarks.—The experiment confirms the results of the previous experiments. All the hoppers exhibited intense *gregaria* coloration in the fourth instar. The intensity of coloration we are inclined to attribute to the density of the crowd of the associates (15). The progress of the black pigmentation from instar to instar is given in Table XII and the life cycle of these hoppers is given in Table XIII.

TABLE XII

Progress of black pigmentation in hoppers in experiment 8

Reference No.	I stage	II stage	III stage	IV stage	V stage	Remarks
P54-57/C1	Green type	Intermediate near green type	......	......	...	Killed and eaten in the II stage by the other hoppers
P54-57/C2	Ditto	Almost green type	......	......	......	Died in II stage
P54-57/C3	Ditto	Intermediate near green type	Intermediate very near green type	Intense black type	......	Died in IV stage
P54-57/C4	Ditto	Black type	Intense black type	Ditto	......	Died in IV stage
P54-57/C5	Ditto	Intermediate near green type	Intermediate very near black type	Ditto	Intense black type.	
P54-57/C6	Ditto	Intermediate near green type	Ditto	Ditto	Ditto	
P54-57/Cont. 1	Ditto	Geren type	Green type	Green type	Green type	
P54-57/Cont. 2	Ditto	Ditto	Ditto	Ditto	Ditto	Control hoppers reared singly
P54-57/Cont. 3	Ditto	Ditto	Ditto	Ditto	Ditto	

TABLE XIII

Life history of hoppers in experiment 8

Date (1932)	Daily temperature (°C.)		Experimental					
	Max.	Min.	P54-57 C1	P54-57 C2	P54-57 C3	P54-57 C4	P54-57 C5	P54-57 C6
14th Sept. . .	34·5	31	I	I	I	I	I	I
15th ,, . .	35	32						
16th ,, . .	34·5	32						
17th ,, . .	35	30						
18th ,, . .	34	30						
19th ,, . .	35	30		II			II	II
20th ,, . .	34	29·2	II		II	II		
21st ,, . .	33·5	29						
22nd ,, . .	33·5	29						
23rd ,, . .	33	29						III
24th ,, . .	33	28·5	IID		III	III	III	
26th ,, . .	32·5	28·4						
27th ,, . .	32·5	28						
28th ,, .	32·5	28						
29th ,, . .	32·5	28·5		IID				
30th ,, . .	33	29						
1st Oct. . .	33	29						
2nd ,, . .	33	29						
3rd ,, . .	32·2	28						
4th ,, . .	32·5	28·5						
5th ,, . .	31·5	27·5						
6th ,, . .	31·5	27						
7th ,, . .	31·5	27						
8th ,, . .	31·5	26·5						
9th ,, . .	30·5	26						
10th ,, . .	30	26						IV
11th ,, . .	29·5	25·5				IV	IV	
12th ,, . .	29·5	25·5						
13th ,, . .	30·5	25·5						
14th ,, . .	30	25						
15th ,, . .	30	25				Died		
16th ,, . .	30	25						
17th ,, . .	30·5	25						
18th ,, . .	31·2	24						
19th ,, . .	31	24						
20th ,, . .	29	23						

TABLE XIII—*contd.*

Life – history of hoppers in experiment 8—contd.

Date (1932)	Daily temperature (C.)		Experimental					
	Max.	Min.	P54-57 C1	P54-57 C2	P54-57 C3	P54-57 C4	P54-57 C5	P54-57 C6
21st Oct. . .	29	23						
22nd ,, . .	29	23						
23rd ,, . .	29	23						
24th ,, . .	29	23						
25th ,, . .	28	22						
26th ,, . .	28	22						
27th ,, . .	28	22						
28th ,, . .	28	21						
29th ,, . .	28	20						
30th ,, . .	21	20						
31st ,, . .	21	20·5			IV			
1st Nov. . .	22·7	20·5			stopped			
2nd ,, . .	21	18·3						
3rd ,, . .	23·8	18·8						
4th ,, . .	23·3	18·8						
5th ,, . .	23·3	18·8						
6th ,, . .	23·3	17·7						
7th ,, . .	23·3	18·3						
8th ,, . .	23·3	17·7						
9th ,, . .	23·3	17·7						
10th ,, . .	22·7	17·7						
11th ,, . .	22·7	17·7						
12th ,, . .	22·7	17·7					V	
13th ,, . .	22·7	17·7					stopped	
14th ,, . .	22·7	18·3						
15th ,, . .	23·3	17·7						
16th ,, . .	22·7	17·7						
17th ,, . .	22·7	17·2						
18th ,, . .	21·6	17·2						
19th ,, . .	22·2	17·3						
20th ,, . .	22·2	16·6						
21st ,, . .	22·2	16·6						V

Experiment 9

The hoppers employed in this experiment were of the '*solitaria*' type.

Associates.—The adults of house cricket (*Gryllus domesticus*) were the associates selected for this experiment. Their strength was maintained between fifteen and twenty in each cage.

Conditions.—The cages employed measured 6 in. × 6 in. × 6 in. and had three of the sides and the top of wire gauze. The bottom was soil and the fourth side was the glass door. Fresh leaves were supplied as food.

The experiment was conducted during the summer of 1931.

Remarks.—The hoppers in this experiment had about the same degree of '*solitaria*' tendency as in experiment (4) and were reared in cages of similar dimensions. The number of associates was about the same as in experiment (8) and dicidedly more than in any of the experiments (5), (6) and (7). The 'associates' in this case were the adults of *G. domesticus* as against *P. pictus* and *Chrotogonus* spp. in other experiments. All other conditions in this experiment were practically the same as in experiments (4), (5), (6), (7) and (8), but the strength of associates was higher. One would have expected a better development of the black pigmentation, but, on the contrary, all the hoppers, instead of developing black pigmentation, continued to maintain their '*solitaria*' coloration. The absence of the appearance of black pigmentation can only be attributed to the distinctly different behaviour of the associates, *G. domesticus*, which being nocturnal are active at night.

V. Discussion

In experiments (1) to (4), in which isolated '*solitaria*' hoppers of *Schistocerca gregaria* were made to take exercise by walking, the black pattern of the *gregaria* phase appeared in spite of isolation. Again in three of the experiments [Experiments (5), (7) and (8)] where single hoppers of *Schistocerca gregaria* were kept in association with other grasshoppers, typical *gregaria* black patterns appeared. In experiment (6) all the hoppers did not show the typical '*gregaria*' coloration and in experiment (9) all the hoppers behaved as if they were living a life of absolute isolation, that is, none of the hoppers showed any signs of *gregaria* coloration. Why this difference ? In the case of experiment (6) we have already hinted that the conditions were near a neutral line, a slight deviation in the environmental conditions on one side or the other resulting in a *solitaria* or *gregaria* coloration.

The explanation for the peculiar results in experiment (9) lies in a different direction. The '*solitaria*' hoppers in this experiment have the largest number of associates, but the associates belong to that class of insects that have habits diagonally opposite to those of the hoppers of the Desert Locust. *Gryllus domesticus* is a nocturnal insect and avoids light. In the cages used in our experiment, it kept itself hidden during the day time, under any lump of soil or below the leaves provided for feeding. Therefore, so far as the question of physical exertion, arising from the frequent contact of the insect under experiment and its associates or from the movement induced through seeing the associates moving about goes, the hoppers of the Desert Locust in association with *G. domesticus*, may, for all practical purposes, be regarded as isolated. Thus, it is not at all surprising that hoppers in such conditions should continue to be unaffected by 'association' and maintain the '*solitaria*' coloration.

This also shows that different insects, as associates, from the view-point of the appearance of '*gregaria*' coloration in the associated hoppers, differ a great deal. The most successful, from this point of view, is the one which is nearest to the hopper of Desert Locust in point of habit and behaviour. It will appear that even *P. pictus* hoppers are not ideal associates, if we bear in mind that in a cage 6 in. × 6 in. × 6 in. the minimum number of *P. pictus* hoppers required is about three whereas even two *Schistocerca gregaria* hoppers bred in such a cage acquire typical '*gregaria*' coloration.

Physical exertion being the main common factor in the experiments detailed above, and in the case of hoppers in a swarm in nature and those reared in laboratory in a crowded condition, where *gregaria* coloration appears, we are justified in concluding that the appearance of black pigmentation can be brought about by muscular activity.

VI. Summary

Two automatic devices, by which isolated hoppers of the Desert Locust were made to take muscular exercise have been described. In the construction of these advantage was taken of: (*a*) positive heliotropism or phototropism and (*b*) negative geotropism of the hoppers.

It has been experimentally proved that an isolated hopper (having '*solitaria*' tendency at the start) develops '*gregaria*' coloration when forced to move through a certain distance each day.

Association with other Orthoptera increases the activity of the hoppers of the Desert Locust with the result that the '*gregaria*' coloration is developed. It has been shown that for this purpose the associates should be akin to the hoppers under experiment in the matter of habit and behaviour. The greater the likeness in this respect, the quicker is the response and the smaller is the number of associates required to bring about the colour change.

Since in the life of hoppers belonging to a swarm or to a crowded laboratory rearing and in the life of the hoppers in the experiments described in this paper, the chief common feature is activity, it is concluded that the black pigment is produced as the result of physical muscular exertions.

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STUDIES ON *SCHISTOCERCA GREGARIA* FORSK.

*VI. INFLUENCE OF TEMPERATURE ON THE INTENSITY AND EXTENT OF BLACK PATTERN IN THE DESERT LOCUST HOPPERS BRED CROWDED

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(Received for publication on 6th January 1936)

(With Plates XXXVI-XL)

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I. Introduction

It is well known that the hoppers of *Schistocerca gregaria* Forsk. exhibit a great range of variation in coloration—from almost pure light green to almost pure black with some yellow patches. Even in the first stage the hoppers exhibit three distinct colour forms. They may have an intense pigmented pattern when they may be termed ' black ', or they may be more or less without pigmentation having light green colour, when they may be termed 'green', or they may have pigmentation less intense and not so extensive, when they may be called ' intermediate '. All through the first instar they retain the pattern and coloration with which they are born. From the same packet of eggs there may appear : (1) a pure black hatching, *i.e.*, all the hoppers are pigmented, (2) a pure green hatching, *i.e.*, all the hoppers are unpigmented, *i.e.*, green, or (3) a mixed hatching, which may consist of (i) black and green, (ii) black, intermediate and green, (iii) black and intermediate, or (iv) green and intermediate. It may be made clear that these colour forms are distinct from the intensification of colour which usually occurs a little time after hatching or after a moult.

Repeated observations, by a number of investigators, on different locusts, have established that the locust hoppers bred isolated or singly, *i.e.*, one hopper in each breeding cage, do not ordinarily develop black pigmented pattern, and, in the absence of pigmentation, the entire coloration of the body is some shade of green. If, on the other hand, more than one hopper are placed together in a small cage, *i.e.*, they are bred crowded, then they develop a black pigment, deposited in a more or less well-marked pattern. The intensity and extent of pigmentation depends, to a certain degree, on the duration of crowding and the density of population. The real cause of this interesting phenomenon is at present not known.

In nature the pigmented forms are characteristic of the swarms and the green forms occur in isolation, although not infrequently green hoppers also occur in the swarms of black hoppers.

During the course of our investigations on the bionomics of *Schistocerca gregaria* Forsk., we have had an opportunity of studying the influence of temperature on the extent of colour pattern and the intensity of pigmentation. The results obtained are very striking and are presented in this paper.

So far no detailed study of the development of pigmentation in different parts of the body of a hopper has been undertaken and vague description in such terms as 'pigmented', 'non-pigmented', 'intermediate', does not convey a precise idea. In this paper, therefore, a full description of the coloration of the hoppers has been given and illustrated by coloured plates. In the descriptions that follow we have employed the usual nomenclature and have introduced certain terms for specific colour patches on various parts of the body (Plate XXXVI).*

We have to acknowledge with thanks the assistance received from the Artist of the Entomological Section, Mr. Siddiqi, in the preparation of these plates.

This investigation was carried out under the scheme of Locust Research financed by the Imperial Council of Agricultural Research. We gratefully acknowledge the generosity of the Council.

II. Experimental technique

The cages employed in these experiments were cylinders of brass wire-gauze (100 meshes to an inch). Each cage was six inches long and two inches in diameter, closed at one end and at the other end it was provided with a split cork through which a cabbage leaf—the food on which hoppers were reared—could be introduced. In all cases young cabbage plants, grown in earthen pots were provided as food and the cages were placed on the growing plant. The pots with the cages were kept in incubators at the required temperature. Thus the hoppers had abundant food always available and it may be assumed that the humidity was always high. There was no special arrangement for ventilation in the incubators, although the space available made the supply of oxygen abundant.

The hoppers were bred at the following constant temperatures :—

24°, 27°, 30°, 33°, 36°, 40° and 44° C.

It may be mentioned that at a temperature below 24° C. and above 44° C., the hoppers were not able to complete their life cycle.

Experiment I

History.—Reference No. L 224.

Both the male and female, the progeny of which was used for this experiment, were obtained from a batch of hoppers consisting of forty black and two intermediate hoppers which had hatched on 11th August 1932. They were bred crowded at room temperature in a wire-gauze cage 9 in.×9 in.×9 in. They acquired wings on 7th September 1932. The females from this batch were fertilized by the males from the same batch. A packet of eggs was laid on 20th October 1932.

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* This plate is hypothetical, meant to illustrate the terms used, otherwise striped eyes and black pigment do not go together.

The eggs hatched on 11th November 1932. On the basis of their coloration the hoppers could be classified as :—

black 42, intermediate 4, green 0.

The hoppers selected for this experiment were pigmented and thus came from a stock showing pigmentation for two generations.

Three hoppers were placed in each cage, and bred at the following constant temperatures :—

24° C. .	2 sets.	27° C.	1 set.	30° C.	2 sets.
33° C.	2 sets.	36° C.	3 sets.	40° C.	1 set.

Experiment II

History.—Reference No. P. 74.

Both the male and female, the progeny of which was employed in this experiment, were solitary adults captured from Sonmiani (South Baluchistan) on 31st October 1932. The pair was placed in a wire-gauze cage 9 in. × 9 in. × 9 in. from the date it was captured and kept in the hot house.

The female laid a cluster of eggs on 29th December 1932 and the eggs hatched on 14th January 1933. The hoppers were :—

black 24, intermediate 6, green 0.

The hoppers selected for this experiment were pigmented. The hoppers of this batch although descended from solitary parents were predominantly melanic.

Three hoppers were enclosed in each cage, and one cage was placed at each of the following constant temperatures :—

24°, 27°, 30°, 33°, 36° and 40° C.

Experiment III

History.—Reference No. L 276.

Hoppers were bred crowded in a muslin cage in the open, yielding *gregarious* adults, which acquired wings on 14th November 1932. From the date of acquiring wings two females and one male were placed together in a wire-gauze cage 9 in. × 9 in. × 9 in. and kept in the hot house.

One female laid a cluster of eggs on 1st January 1933. The eggs hatched on 16th January 1933 and produced fifty-five black hoppers. These hoppers again showed marked pigmentation.

In each cage seven hoppers were kept and one cage was placed at each of the following constant temperatures :—

24°, 27°, 30°, 33°, 36° and 40° C.

(For the duration of life-cycle, mortality, etc., see Tables I, II and III).

III. Body coloration of hoppers of different stages

1. DESCRIPTION OF THE COLORATION OF TYPICAL 1 STAGE BLACK HOPPERS (PLATE XL, FIG. I)

(a) *General colour*

Black predominates, dirty yellowish white appearing at places only. A dorso-lateral yellowish streak starting from behind the compound eyes extends over thorax and abdomen, becoming narrower caudad. On pronotum this stripe extends along the caudal margin and forms a wide comma-shaped whitish area.

(b) *Head*

Frons.—Black, right up to the bases of antennae and beyond.

Clypeus.—Black, except for greyish dirty white along the ventral and lateral margins.

Labrum.—Black, except the notch.

Vertex.—Black on both sides of the dirty white epicranial suture, black colour continues behind the eyes on the genae and is continuous with the black sub-ocular region, leaving a post-ocular patch, which is dirty white suffused with yellowish tinge.

Genae.—Entirely black, sub-ocular genal stripe, therefore, not distinguishable.

Postgenae.—Black.

Compound eyes.—Dark chocolate-brown, excepting a narrow dirty pale yellow stripe along the entire mesal margin. Not striped.

Antennae.—Black, intersegmentally dirty white, basal joints also black.

Mandibles.—Black.

Maxillae. }
Labium. }—Black, palpi intersegmentally dirty white.

(c) *Neck*

Cervical sclerites.—Black.

(d) *Thorax*

Pronotum.—Keel with a white median line and narrow black longitudinal stripes on each side of it, the latter widen towards the caudal portion forming a small metazonal triangle on each side. Rest of the pronotum black except a dirty yellowish white hoop or comma-shaped patch which runs cephalo-caudad, parallel to the keel along the black stripe above mentioned and then curves down along the caudal edge of the pronotum and curves again and runs along the ventral margin.

Meso— and meta-nota.—With a mid-dorsal yellowish white line in continuation of the pronotal keel ; wider black stripes on both sides of it, in continuation of the similar black stripes on pronotum, bounded by lateral dirty yellow stripes on each side ; entire lateral area below these yellowish stripes black.

Meso-episterna
Meso-epimera
Met episterna
Met-epimera
}.—Mostly black with small yellowish spots.

Sterna.—Mostly black with dirty yellow ground colour appearing at places.

(*e*) *Legs*

I and II.—Dirty yellow with numerous smoky dots giving a general smoky appearance ; dots fused at the tibio-femoral joints ; claws black.

III.—Hind femurs : entire anterior as well as posterior muscle-impression areas black ; the black pattern meets dorsally leaving an ill-defined spot of basic colour on the dorsal carina of the femur ; apex of femur black and between this and the black pattern on the body of the femur intervenes a dirty yellow ring.

Tibial spines black.

(*f*) *Abdomen*

Segments-I-X.—Each tergum has a black mid-dorsal stripe with a small ivory coloured mid-dorsal triangle at the caudal margin, which becomes successively fainter and smaller on the hinder segments. Along this black stripe, and running parallel to it on each side, is a dirty white stripe which becomes much narrower on the hinder segments and becomes almost invisible on the last segments. Below this the entire tergal area appears black with the exception of an ivory coloured oblique streak on each segment. In the last three segments the oblique white streak is not distinguishable. Below the oblique streak is, so to say, a continuous black stripe and finally along the margin of the tergum is the yellowish white spiracular stripe.

Telson and *cerci*.—Almost black.

Ventral surface.—Black, with a smoky mid-ventral line.

The above is the description of the coloration of the first stage black-pigmented hoppers which were the starting point of all the experiments described below.

2. DESCRIPTION OF THE COLORATION OF THE SECOND STAGE HOPPERS

i. *Coloration of II stage hoppers bred crowded at 24° C.* (*Plate XXXVII, fig. 1*)

(*a*) *General colour*

Black and dirty white ; lateral whitish streak along the side extending on thorax and abdomen. A comma-shaped whitish area on the pronotum.

(*b*) *Head*

Frons.—Black predominates : entire frons up to the bases of antennae black, rest dirty white with faint pinkish tinge.

Clypeus.—Black.

Labrum.—Black.

Vertex.—Black triangular areas on each side of a fine yellowish-orange epicranial suture ; black colour is continuous behind eyes and joins the black subocular region, leaving a very small yellowish-orange post-ocular patch.

Genae.—Entirely black, sub-ocular genal stripe, therefore, not distinguishable.

Postgenae.—Black.

Compound eyes.—Uniformly dark reddish-brown ; not striped.

Antennae.—Black, smoky intersegmentally.

Mandibles.—Black.

Maxillae.
Labium. } .—Dull white with black patches, palpi black.

(*c*) *Neck*

Cervical sclerites.—Black.

(*d*) *Thorax*

Pronotum.—Keel, anterior 3/4 black, posteriorly white ; narrow dark black stripes on both sides of keel fused towards the cephalic portion and become wider forming triangular areas caudally ; similarly on caudal portion they form the metazonal triangular patches ; lateral aspect of pronotum black, except for a hoop of comma-shaped white patch, wide dorsad and caudad and gradually becoming narrower and whiter ventrad.

Meso- and Meta-nota.—Mid-dorsal whitish line, with black stripe on either side of it, and further down ; below this a dirty white straight stripe and a black stripe ; angle from where the wing pads arise black.

Meso-episterna.—Brown at the margins, brownish black in the middle.

Meso-epimera.—Brownish black.

Met-episterna
Met-epimera } .—Black.

Sterna.—Uniformly brownish black.

(*e*) *Legs*

I.—Dorsally almost all black, but ventrally white ground colour appears at places. Claws black.

II.—Dull white with numerous black dots, black along the joints.

III.—Hind femurs : almost the entire muscle-impression areas on the anterior as well as posterior faces black, with a very faint small patch of yellowish colour on the dorsal carina just about the middle. This black pattern almost fused with a wide black ring round the apex of femur.

Base and apex of tibia black ; spines, tarsi and claws black. A few whitish dots on tarsi.

(*f*) *Abdomen*

Segment I.—Mid-dorsal pigmented stripe black and fairly wide, white triangle at posterior margin reduced. Laterally outline of ear black with eight dots irregularly arranged above it.

Segments II-VIII.—Laterally, below the mid-dorsal black stripe is a narrow straight white stripe running through all the abdominal segments except the last three. The tergal area below this stripe and above the white spiracular stripe looks black on account of the fused masses of black spots. There is an oblique white streak running diagonally on each segment.

Segment IX.—Black laterally, only a white dot at each dorso-lateral corner.

Segment X.—All black.

Terminal portion.—Brownish black.

Ventral surface.—Uniformly brownish black with a whitish mid-ventral streak.

(*ii. Coloration of II stage hoppers bred crowded at 33° C.* (*Plate XXXVIII, fig. 1*)

(*a*) *General colour*

Dull light yellow tinge predominates, although black pattern also well marked (but not so well developed as in the hoppers bred at 24° C.).

(*b*) *Head*

Frons.—Dull light yellow with fine black dots ; lower portion black, getting fainter above.

Clypeus.—Irregular, blurred black spots ; dorsal portion lighter.

Labrum.—Black, lighter at the emargination.

Vertex.—Black on both sides of the fine white epicranial suture. Black colour creeping behind the eyes. Post-ocular patch brownish orange, extensive, as long as or longer than the compound eye.

Genae.—Sub-ocular genal stripe black, very wide and extends up to the inner margin of the eyes. Area behind this stripe brown.

Postgenae.—Light brown to black.

Compound eyes.—Dark reddish brown, without any stripes : anterior half with a greyish tinge.

Antennae.—Black ; basal one segment whitish. White intersegmentally.

Mandibles.—Black.

Maxillae }
Labium } .—Mixed smoky and white.

(*c*) *Neck*

Cervical sclerites.—Black but lighter in colour.

(*d*) *Thorax*

Pronotum.—Keel white, narrow black stripes along its each side getting wider at the anterior margins forming anterior triangles. Metazonal triangular patches black, not extensive. Lateral black patches very large, covering more than half of prozona leaving a broad comma-shaped white patch running dorsal and cephalad.

Meso- and *meta-nota.*—Dorsal black stripe on both sides of the white median line ; below this a white straight stripe and then a broad black straight stripe.

Meso-episterna
Meso-epimera } .—Yellowish white with black dots.

Met-episterna
Met-epimera } .—Black.

Sterna.—White with large black patches.

(*e*) *Legs*

I and II.—Dull yellowish white, with black dots throughout. Black colour along the joints.

III.—Hind femur : black pattern on the anterior as well as posterior muscle-impression areas, with a small oval gap on the dorsal carina, which runs a little each way on the anterior and posterior aspects. The black pattern not fused with the black ring round the apex of femur.

Both the ends of tibia black ; spines also black.

(*f*) *Abdomen*

Segment I.—A wide dark black stripe dorsally with a white triangular area at posterior margin. Laterally six to seven black dots irregularly arranged.

Remaining segments.—Mid-dorsally, in each segment a wide black stripe with white triangular area at posterior margin; laterally five black dots along the posterior margin ; in front of these a straight black stripe and above it an oblique black streak and a number of (usually four) black dots dorsad to this.

Terminal part of abdomen.—The black pattern distinctly reduced as compared to the hoppers at 24° C.

Ventral surface.—White with large smoky dots.

iii. Coloration of II stage hoppers bred crowded at 40° C. (*Plate XXXIX, fig. 1*)

(*a*) *General colour*

Much lighter than the hoppers at 33° C., but with more of pigment than in the hoppers bred at 44° C. General colour dull whitish with a yellowish tinge.

(*b*) *Head*

Frons
Clypeus } .—Uniformly yellowish white without any smoky dots.
Labrum

Vertex.—Broken smoky areas on both sides of the epicranial suture ; rest very light brown.

Genae.—Sub-ocular genal stripe narrow ; smoky regions in front and behind, dull white.

Postgenae.—Very light brown, almost whitish.

Compound eyes.—Anterior half, light grey ; posterior, dark reddish brown. Not striped.

Antennae.—Very light smoky, whitish intersegmentally.

Mandibles.—White, dentation black, smoky near labium and clypeus.

Maxillae }
Labium } .—White with faint smoky marks on palpi.

(*c*) *Neck*

Cervical sclerites.—Light smoky.

(*d*) *Thorax*

Pronotum.—General colour white, with a yellowish tinge. Keel whitish, very narrow smoky stripes on each side. Lateral patch large brownish, its prozonal portion is mixed smoky and white, and metazonal portion smoky brownish, much reduced. Metazonal triangular patches brownish black. The Comma-shaped area very extensive.

Meso- and meta-nota.—Dorsal brownish black stripe on both sides of white median line, whitish areas below this followed by the brownish black stripe.

Meso-episterna }
Meso-epimera } .—White with two faint black dots.

Met-episterna.—White ; a narrow smoky streak at the posterior margin.

Met-epimera.—White ; a narrow smoky streak at the anterior margin fused with a smoky streak at the posterior margin of met-episternum.

Wing pads.—Smoky.

Sterna.—Uniformly white.

(*e*) *Legs*

I and II.—Dull white ; pinkish marks at the basis of hairs. Smoky marks close to the joints.

III.—Hind femur : a narrow smoky pattern on the anterior muscle-impression area and a similar pattern on the posterior muscle-impression area, leaving a small ring of dull white with yellowish tinge between this pattern and the black colour round the apex of femur. The pattern on the anterior and posterior aspect of the femur fused dorsally except for a broadly oval area on the dorsal carina.

Apex of femur, and base and apex of tibia brownish black. Tarsi brown, claws light black.

(*f*) *Abdomen*

Segment I.—Dorsally light black stripe with a white triangular area at the posterior margin ; 4 smoky dots on the lateral region.

Remaining segments.—Dorsally as in segment I, laterally five brown dots along the posterior margin. In front of these, ventralmost is a straight broken brownish stripe ; above this an oblique brown streak and dorsal to it two to three light black dots.

Terminal portion.—Yellowish white.

Ventral surface.—Dull white without any dots.

*iv. Coloration of II stage hoppers bred crowded at 44° C. (Plate XL, fig. 2)

Entire body colour almost whitish.

Eyes colourless or light greyish, except dorsal lunar area which is dark. Antennae colourless.

Claws and spines very light reddish brown at tips, but tarsi white. A few scattered dots on the femur, but no trace of the black pattern. Lunar ridge on trocolia, very light brown.

3. DESCRIPTION OF THE COLORATION OF THE THIRD STAGE HOPPERS

i. Coloration of III stage hoppers bred crowded at 24° C. (Plate XXXVII, fig. 2)

(*a*) *General colour*

Black predominating, rest dirty white with an almost imperceptible yellow tinge.

(*b*) *Head*

Frons.—Dorsally dirty to light brown; lower portion black; black colour creeping up and gradually fading.

Clypeus.—All black.

Labrum.—Black except near the emargination.

Vertex.—Epicranial suture very faint; black area on each side, black colour creeping down behind the compound eyes, leaving a small yellowish brown post-ocular patch.

Genae.—Entirely black; sub-ocular genal stripe not distinguishable.

Postgenae.—Black.

Compound eyes.—Not striped; dark reddish brown; anterior margin lighter (dirty white).

Antennae.—Light smoky colour on the basal joint, rest black; whitish line along the lateral aspect; terminal joints lighter.

Mandibles.—Black.

Maxillae.—Black; palpi also black except terminal portion of joints which are lighter.

(*c*) *Neck*

Cervical sclerites.—Black.

(*d*) *Thorax*

Pronotum.—Pale yellow dots all over the pronotum. Anterior margin whitish. A black stripe on the keel throughout, except anterior end. The triangular metazonal patches black fused in the middle. Lateral black patch very large, separated from median line and metazonal patch by a dull comma-shaped band.

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* The size of the hoppers bred at 44° C. is below the normal. This reduction is more marked in advanced stages. This is clearly seen in Plate XL, figs. 2—5.

Meso- and meta-nota.—Mid-dorsal black stripe in continuation of the black keel of the pronotum. Below this straight white stripe on each side.

Meso-episterna.—Light fawn, with smoky dots.

Meso-epimera.—Dirty white with light yellowish spots.

Met-episterna
Met-epimera } .—Black.

Wing pads.—Entirely black.

Sterna.—Smoky ; presternum black.

(*e*) *Legs*

I and II.—Coxae and trochanters smoky ventrally and at some other places. Femur brown to black dorsally towards apex. Tibia, head and apex black, anterior aspect black. Tarsi black, less black in II pair.

III.—Hind femur : anterior muscle-impression area entirely black except near base and apex ; its distal end extends dorsally and posteriorly with an ill-defined gap between, and ends just above the postero-ventral femoral carina. Apex of femur black.

Head and apex of tibia black. Spines black. Tarsi dorsally black.

(*f*) *Abdomen*

Segment I.—Black median stripe wide, with white triangular area. Laterally two rows of irregular spots on dirty white ground colour.

Segments II-VIII.—Dorsally as in segment I, laterally brown to black stripe just above the white spiracular stripe, then well-marked oblique black streak, and two rows of black irregular spots above this.

Segment IX.—Laterally dark except a very small dirty white patch.

Segment X.—Entirely black.

Telson.—Smoky with black spots.

Ventral surface.—Brownish to black areas on both sides of a whitish median streak.

ii. Coloration of III stage hoppers bred crowded at 33° C. (*Plate XXXVIII, fig.* 2)

(*a*) *General colour*

Dull white with a pinkish and yellowish tinge.

(*b*) *Head*

Frons.—Pink, dirty white at places ; two black dots at the lower end of each frontal carina.

Clypeus.—Dull white with a greyish tinge, black along ventral margin.

Labrum.—Black, with dull white spots at places.

Vertex.—Black areas on both sides of epicranial suture which is a fine yellowish orange line. Black colour continued down behind the eye, leaving an orange-yellow post-ocular patch.

Genae.—Dull white in front of the sub-ocular genal stripe, which is a wide black streak creeping up behind the eye, but here it is blurred black.

Post-genae.—Dull white.

Compound eye.—Striped ; three reddish brown stripes, almost equally wide, with whitish interstripes.

Antennae.—Black, basal segments white, and whitish along the lateral aspect.

Mandibles.—Anteriorly black, laterally brown, posteriorly smoky.

Maxillae
Labium } .—Dull white, palpi black and white.

(c) *Neck*

Cervical sclerites.—Smoky.

(d) *Thorax*

Pronotum.—Basic colour dull white with a pinkish tinge and pale yellow dots. Light pink colour along the sides of keel, which has a fine white line with a narrow black stripe along each side. These black stripes become broader anteriorly and posteriorly ending in triangular metazonal patches. Lateral black pattern, lighter in the prozonal region, dark black in metazonal region. Intervening comma-shaped patch broad and white with a pinkish tinge particularly in the anterior half of it.

Meso- and meta-nota.—Mid-dorsal stripe black and in continuation of the black keel of the pronotum ; below this straight white stripes with a pinkish tinge.

Meso-episterna
Meso-epimera } .—Light yellow.

Met-episterna.—Dorsal half black, ventral dull white with a vertical black line in the middle.

Met-epimera.—Black.

Wing pads.—Black.

Sterna.—White with black dots.

(e) *Legs*

I and II.—Dull white with dark-brown dots. Claws light black. Femoro-tibial joints brownish, the brown colour going each way about 1/8th length of femur and tibia. Spines and claws black ; spurs black with brown ends.

III.—Hind femur ; black pattern on the anterior muscle-impression area covering dorsal 2/3 of entire surface except (i) about 1/4 of its proximal end between the antero-dorsal carina and the muscle-impression area and (ii) a small gap on the dorsal margin of distal end. The two extensions of the pattern on either side of the gap grow wider as they are continued to the posterior side and end on the posterior muscle-impression.

(*f*) *Abdomen*

Segment I.—A wide black dorsal median stripe, with a white triangle at the posterior margin, the vertex of which is continued anteriorly in short distances as a fine white line. Laterally three dorso-ventral rows of dots; three dots in the posterior row, two in the middle and one to two in the anterior.

Segments II-VIII.—Dorsal stripe as in segment I. Laterally six dots along posterior margin; dorsal two dots almost touching the dorsal stripe. In front of the ventralmost dot is a straight black stripe, above it the oblique streak and over this three-four dots. Basic colour dull white.

Segment IX.—Laterally only a black line along its posterior margin.

Segment X.—Laterally scattered black spots; terminal portion dull white.

Ventral surface.—White with black dots.

iii. Coloration of III stage hoppers bred crowded at 40° C. (*Plate XXXIX, fig. 2*)

(*a*) *General colour*

White with a slight yellowish tinge.

(*b*) *Head*

Frons
Clypeus }.—Uniformly pale yellowish.
Labrum

Vertex.—Narrow black streaks on each side of the epicranial suture which is light brown; post-ocular patch extensive orange-yellow.

Genae.—Whitish yellow in front and light orange-yellow behind the narrow smoky sub-ocular genal stripe.

Postgenae.—Very light brown, almost whitish in places.

Compound eyes.—Striped; four chocolate-brown stripes visible with whitish grey inter-stripes.

Antennae.—Smoky in the middle, whitish towards the distal and basal ends.

Mandibles.—Exposed parts whitish, dentation black.

Maxillae
Labium }.—White, faint black dots on palpi.

(*c*) *Neck*

Cervical sclerites.—Smoky.

(*d*) *Thorax*

Pronotum.—Pale yellow; keel whitish behind the hind sulcus. Reduced black pattern in the prozonal region; blurred brownish-black patch laterally in metazonal region. The metazonal patches, triangular in form on each side of median line, are brownish black with whitish dots.

Meso- and meta-nota.—Mid-dorsal black stripe in continuation of the black keel of the pronotum. Below this there is a straight white stripe.

Meso-episterna
Meso-epimera }.—Uniformly pale yellow.

Met-episterna.—Pale yellow ; blurred black spots along posterior margin.

Met-epimera.—Pale yellow ; blurred black spots along anterior margin.

Wing pads.—Black dots on the base as well as on the pad.

Sterna.—Pale yellow without any dots.

(e) *Legs*

I and II.—Dull white without black markings.

III.—Hind femur ; black pattern on the anterior muscle-impression area not wide and diffused. Its dorsal margin is separated from the antero-dorsal carina by a wide streak of basic colour except near the distal end where two narrow extensions of the pattern run dorsally, enclose a wide gap of ground colour and end on the postero-dorsal carina.

(f) *Abdomen*

Segment I.—Tergum has dorsally a light black narrow stripe with a large white triangular area at the posterior margin ; four to five black dots on each lateral aspect.

Segments II-VIII.—Tergum dorsally as in segment I. Laterally a row of five black dots along posterior margin. Opposite to the lowest dot is a smoky straight stripe, above this an oblique smoky streak and over this three smoky dots parallel to the oblique streak.

Segments IX-X.—Tergum laterally with only a row of black dots, fused into a line along the posterior margin.

Genital appendages.—Light yellow.

Ventral surface.—Yellow with faint brownish dots.

iv. Coloration of III stage hoppers bred crowded at 44° C. (Plate XL, fig. 3)

Body colour whitish with a pale yellowish tinge.

Eyes whitish anteriorly, darker along the posterior margin. Lunar area of the eye dark. Antennae white.

Frons and mouth-parts white, post-ocular region pale yellow. Legs whitish, dull yellow on the anterior surface of hind femur.

Abdomen white with a pale yellow tinge ; brownish dots on the lateral aspect.

4. DESCRIPTION OF THE COLORATION OF THE FOURTH STAGE HOPPERS

i. Coloration of IV stage hoppers bred crowded at 24° C. (Plate XXXVII, fig. 3)

(a) *General colour*

Black predominating ; ground colour dirty yellow with a mixed green and pinkish tinge.

(*b*) *Head*

Frons.—Dull yellow ; ventral about 1/8th black, black colour getting fainter upwards. Numerous black dots along the carinae.

Clypeus.—Black.

Labrum.—Black, notch lighter.

Vertex.—Epicranial suture not distinct. Entire area behind eyes black, joining the sub-ocular black area, except a very small orange-red post-ocular patch.

Genae.—Black ; sub-ocular genal stripe, therefore, not distinguishable.

Post-genae.—Black.

Compound eyes.—Not striped ; uniformly dark reddish brown.

Antennae.—Dark black except the basal two segments which are dirty yellow. Lateral aspect of antennae lighter.

Mandibles.—Black.

Maxillae, *Labium* }.—Black. Palpi intersegmentally whitish.

(*c*) *Neck*

Cervical sclerites.—Black.

(*d*) *Thorax*

Pronotum.—Entire pronotum, except a reduced ‘comma-shaped’ patch, is black with yellow specks and spots. This patch intervenes between the triangular metazonal patch and the rest of black prozona. White along the ventral margin as well as lower part of anterior and posterior margins. Keel white behind the posterior sulcus, black in front of it.

Meso-episterna.—Dull deep brown ; irregularly black along the anterior margin.

Meso-epimera.—Dull deep brown ; black along dorsal and posterior margin.

Met-episterna.—Black.

Met-epimera.—Black.

Wing pads.—Base black, black lines along veins.

Sterna.—Numerous irregular black patches on a dirty brown ground colour.

(*e*) *Legs*

I and II.—Dull yellow, black ventrally. Femur with dorsal distal half black. Tibia black at both the ends ; spines black. Tarsi dorsally black, ventrally brownish.

III.—Hind femur : the anterior muscle-impression area entirely black with two dark wide extensions at its distal end enclosing a very narrow area of basic colour. The two arms get wider on the muscle impression area of the posterior aspect and end on the postero-ventral carina. Distal end of femur black leaving a ring of basic colour between.

Tibia black at both ends. Spurs black ; tarsi brownish black with black spots ; claws black.

(*f*) *Abdomen*

Segment I.—Tergum covered under wing pads. Dorsal black stripe wide with a white triangular area at the posterior end. Laterally three dots along the posterior margin, a few irregular black dots in front.

Segments II-VIII.—Mid-dorsal stripe as in segment I ; laterally along ventral margin a straight wide black stripe ; above this an oblique black streak over this three to four black dots parallel to the oblique streak ; two dots above this towards posterior margin.

Segment IX.—Tergum dorsally as above, laterally just a line of white colour below the dorsal stripe. Below this all black except a few whitish dots.

Segment X.—Tergum all black. Cerci smoky.

Ventral surface.—Numerous black dots on a dirty brown basic colour, except a yellow streak running mid-ventrally along the length of abdomen.

ii. Coloration of IV stage hoppers bred crowded at 33° C. (Plate XXXVIII, fig. 3)

(*a*) *General colour*

Light yellow with a greenish tinge predominates.

(*b*) *Head*

Frons.—Bright yellow ; a few brown dots near the ventral margin.

Clypeus.—Dull yellow ; dark brown area in the middle and along the lateral margins.

Labrum.—Almost all black.

Vertex.—A triangular black area on each side of epicranial suture. Each side of triangle continued as a black line behind eye and joining the black subocular genal stripe. Post-ocular region brownish orange, extensive.

Genae.—Sub-ocular genal stripe black with a small yellow triangular area in front.

Post-genae.—Black.

Compound eyes.—Striped, with three dark reddish brown stripes. Posterior stripe widest covering half of eye ; the other two stripes are narrow streaks.

Antennae.—Smoky black excepting basal two segments. Intersegmentally faintly whitish.

Mandibles.—All black, except a small yellow spot at base.

Maxillae / *Labium* }.—Pale yellow ; palpi black, only at places with whitish basic colour.

(*c*) *Neck*

Cervical sclerites.—Smoky.

(*d*) *Thorax*

Pronotum.—Greenish yellow with brighter spots, white along posterior and ventral margins. Keel straight from posterior margin to hind sulcus ; slightly convex in front of it. Black in front of hind sulcus, yellow behind it. Lateral black pattern faint in the prozonal region dark in the metazonal region, bent forward at the top. Metazonal triangular patches black with yellow dots.

Meso-episterna.—Uniformly yellow with a greenish tinge. Dorsal margin smoky.

Meso-epimera.—Yellow with a greenish tinge and brighter dots. Dorsal margin smoky. Pleural suture smoky.

Met-episterna.—Greenish yellow ; black patches mixed irregularly ; intenser black along posterior margin.

Met-epimera.—All black, blackness creeping up to the base of the wing pads.

Wing pads.—Base black ; black dots and lines on wing veins.

Sterna.—Yellow with black dots.

(*e*) *Legs*

I and II.—Greenish yellow, light black colour on temur and tibia at the tibio-femoral joint, also at the tibio-tarsal joint. Tarsi dark brown, claws and spines black.

III.—Hind femur : the black pattern on the anterior muscle-impression area wide with two dark black extensions at its distal end going dorsally and ending in the middle of the posterior muscle-impression area. The mesal arm is darker and broader than the other.

Both the ends of tibia light black. Spurs black. Tarsi greenish ; claws deep brown.

(*f*) *Abdomen*

Segment I.—Tergum covered by wing pads. Dorsal stripe faint blackish with a white patch at posterior margin. Four black dots along the posterior margin dorso-ventrally.

Segments II-VIII.—A wide black mid-dorsal stripe with white triangular patch near posterior margin. Five broad black spots along posterior margin ; the ventralmost fused with a fairly wide straight black stripe, the 2nd is fused with an oblique black streak. Against the 3rd dot, there are three dots parallel to the oblique streak, one dot opposite to 4th ; the 5th fused with the dorsal stripe.

Segment IX.—The lateral pattern consists of one wide black streak.

Segment X.—Black.

Telson.—Greenish yellow.

Ventral surface.—Greenish yellow with smoky dots.

iii. Coloration of IV stage hoppers bred crowded at 40° C. (Plate XXXIX, fig. 3)

(*a*) *General colour*

Light yellow.

(*b*) *Head*

Frons
Clypeus }.—Whitish yellow (without any dots).
Labrum

Vertex.—Smoky along both sides of the epicranial suture. Post-ocular patch brownish orange.

Genae.—Pale yellow in front and light orange-yellow behind the sub-ocular genal stripe, which is narrow and slanting.

Post-genae.—Very light yellow (almost whitish).

Compound eyes.—Striped ; four narrow, light chocolate-brown stripes with almost whitish broad interstripes.

Antennae.—Smoky in the middle, whitish at the distal and basal ends.

Mandibles.—Exposed parts yellow, dentation black.

Maxillae
Labium } —Pale yellow.

(*c*) *Neck*

Cervical sclerites.—Smoky.

(*d*) *Thorax*

Pronotum.—Bright yellow, white along the posterior margin. A very fine white streak on keel. On the lateral aspect of prozona a faint smoky patch with yellow dots. The metazonal triangular patches much reduced ; black with bright yellow dots.

Meso-episterna
Meso-epimera }.—White with a yellow tinge. Pleural suture and dorsal margin smoky.

Met-episterna.—Yellow ; black at places, particularly toward the anterior margin.

Wing pads.—A black dot at base, rest pale yellow, with remnants of black lines on veins.

Sterna.—Whitish with a few fine dots.

(*e*) *Legs*

I and II.—Uniformly pale yellow, except tarsi which are laterally faint smoky. Claws and spines brown.

III.—Hind femur : black pattern on the anterior aspect covering only the dorsal 2/3 of the muscle-impression area and separated from the antero-dorsal carina by a wide streak of basic colour, except the two narrow smoky extensions at its distal end enclosing a wide gap of basic colour. The pattern is extremely faint and narrow (as if almost broken) at the ventral end of the gap. The two extensions are continued to the posterior aspect, but do not cover the posterior muscle-impression area and end a little below the postero-dorsal carina.

Spurs smoky ; claws light brown. Spines black tipped ; black dots on terminal end of femur.

(*f*) *Abdomen*

Segments I-X.—Tergum of segment I covered by wing pads ; dorsal black stripe irregular in segment II and III, in others it is present as elongated patch with a white triangular area at the posterior margin. In the lateral aspect there are five to six smoky dots along posterior margin. A narrow straight smoky stripe opposite to the ventralmost, above this an oblique streak and over this five to six black dots.

Telson.—Yellow.

Ventral surface.—Yellow with a few faint smoky dots.

iv. Coloration of IV stage hopper bread crowded at 44° C. (*Plate XL, fig. 4*)

General colour white.

Eyes white with two very fine, light brown stripes ; lunar area dark. Sub-ocular genal stripe extremely faint smoky ; light brownish along the posterior margin in the post-ocular region. Rest of face and head white. Antennae white.

Pronotum almost white.

Legs almost white.

Abdomen whitish with grey colour at places. Fine brownish dots along posterior margin of some of the abdominal segments.

5. DESCRIPTION OF THE COLORATION OF THE FIFTH STAGE HOPPERS

i. Coloration of the V stage hoppers bred crowded at 24° C. (*Plate XXXVII, fig. 4*)

(*a*) *General colour*

Black predominating, with light yellow, pinkish and greenish tinge at places.

(*b*) *Head*

Frons
Clypeus }.—All jet black.
Labrum

Vertex.—All black, except a small brownish portion dorsad of the base of antennae. Post-ocular patch brownish, very much reduced, and concealed under pronotum. Epicranial suture very faint.

Genae.—All jet black, sub-ocular genal stripe not distinguishable.

Compound eyes.—Dark, reddish-brown. No stripes.

Mandibles
Maxillae }.—Black.
Labium

(c) *Neck*

Cervical sclerites.—Black.

(d) *Thorax*

Pronotum.—Entire pronotum jet black ; dotted with very small greenish yellow spots. Sulci along the dorsal portion have pinkish tinge. No separate metazonal patches.

Meso-episterna.—Jet black, with three very small insignificant yellowish dots.

Meso-epimera.—Black with larger and more prominent yellow dots.

Met-episterna.—Jet black, two small yellow dots.

Met-epimera.—Jet black, three small yellow dots.

Wing pads.—Base black, all veins black, rest of wing greenish yellow.

Sterna.—Brownish black.

(e) *Legs*

I and II.—Pinkish brown with black colour over the greater area.

III.—Hind femur : anterior muscle-impression area completely black and fused with the black pattern on the posterior muscle-impression with the exception of the proximal end and a narrow gap of ground colour near the distal end. The posterior black pattern ends on the postero-ventral femoral carina.

Apex and head of tibia jet black, rest dirty orange-brown. Tips of spines, spurs, and dorsal tarsi, black.

(f) *Abdomen*

Segment I.—Tergum covered under wing pad, brownish.

Segments II-VIII.—Mid-dorsal stripe very wide, and black with posterior whitish triangular area. Above the white spiracular stripe is a broad black stripe. Above this an oblique streak which is most prominent and, going up along the interior margin, meets the dorsal stripe. Over this two broken streaks. Very small area of the abdomen appears greenish yellow and even this is suffused with the brown.

Segments IX and X.—Tergum dark black.

Telson.—Yellowish brown.

Ventral surface.—Brownish black with a lighter mid-ventral line.

ii. Coloration of V stage hoppers bred crowded at 33° C. (Plate XXXVIII, fig. 4)

(a) *General colour*

Brownish yellow.

(*b*) *Head*

Frons.—Brownish yellow, darker along the carinae. Few fine black dots close to the ventral margin. Two dots near the vertex.

Clypeus.—Dark brownish yellow ; darker near ventral margin ; black oblique line coming from each dorso-lateral corner.

Labrum.—Black ; a light brown streak exactly in the middle ; lighter along lateral margin, light brown near the notch.

Vertex.—Black on either side of a very faint epicranial suture. The black colour continued down behind the eyes to the sub-ocular genal stripe. Post-ocular area orange.

Genae.—Sub-ocular genal stripe black. In front of it and behind it a yellow area with black dots.

Post-genae.—Pale yellow, with blurred black colour along anterior margin.

Compound eyes.—Five reddish brown stripes. Interstripal area between anterior three stripes whitish, between others light reddish brown, so that stripes are just distinguishable.

Antennae.—Black ; basal two segments and lateral aspect whitish.

Mandibles.—Anterior and posterior aspect black, laterally black with a light yellowish area.

Maxillae } .—Dull white, palpi black, intersegmentally yellowish. Tip of
Labium } galea black.

(*c*) *Neck*

Cervical sclerites.—Black.

(*d*) *Thorax*

Pronotum.—White along ventral and posterior margin. Keel black on the prozonal region and yellow in the metazonal region. Lateral black pattern large, well marked in the metazonal region, blackish brown with yellow spots in the prozonal region. Between the black triangular metazonal patch and the lateral pattern is a brown to yellow comma-shaped band.

Meso-episterna.—Uniformly brown.

Meso-epimera.—Brown with brighter dots.

Met-episterna.—Black, irregular whitish patches at places.

Met-epimera.—Uniformly black.

Wing pads.—Base all black ; black continuous lines on the anterior veins ; black dots on posterior veins.

Sterna.—Yellow with fine smoky dots.

(*e*) *Legs*

I and II.—Brown, a black spot at the apex posteriorly, black dots near the femoral end of tibia. Tarsi light brown ; claws blackish.

III.—Hind femur : a wide black stripe on the anterior muscle-impression area leaving only a narrow line of ground colour above the antero-ventral femoral carina.

Its two dorsal extensions on the distal end are brownish black, and enclose a gap of ground colour almost as wide as the thickness of each extension.

On the posterior aspect the mesal extension is broader and, crossing the posterior muscle-impression area, ends a little above the postero-ventral carina. Distal arm ends a little below the postero-dorsal carina. Apex of femur black.

The head of tibia black ; spines brownish. Spurs dark brown. Tarsi and claws brown.

(*f*) *Abdomen*

Segment I.—Tergum covered under wing-pads, faint markings.

Segments II-VIII.—Mid-dorsal stripe broad, five dots along posterior margin, the lowermost fused with the ventral straight stripe. In front of the 2nd a broken oblique streak. In front of the 3rd and 4th dots, a number of dots irregularly situated. The 5th dot fused with the dorsal stripe.

Segments IX-X.—Tergum dark brown.

Ventral surface.—Yellow, with black dots.

(*iii. Coloration of V stage hoppers bred crowded at 40° C.* (*Plate XXXIX, fig. 4*)

(*a*) *General colour*

Bright yellow, with a slight greenish tinge ; very little of black pigmentation.

(*b*) *Head*

Frons.—Bright yellow, slightly greenish along frontal carinae, no black dots.

Clypeus.—Dorsal half bright yellow, ventral dull white with a yellowish tinge.

Labrum.—Yellow in the middle, dull white along the lateral margins.

Vertex.—Blackish triangular areas on each side of the epicranial suture. A yellow line between compound eye and the above area. Post-ocular patch orange-yellow. The black coloration does not fuse with sub-ocular genal stripe.

Genae.—Yellow, both in front of and behind the black sub-ocular genal stripe, which tapers downwards and is not continued behind the eyes.

Post-genae.—Yellow.

Compound eyes.—Five-six light chocolate-brown stripes with whitish interstripes.

Antennae.—Basal and distal segments whitish or light brown ; middle smoky; whitish along the ventral side.

Mandibles.—Yellow ; anterior margin black.

Maxillae }
Labium }.—Pale yellow ; tips of palpi smoky.

(c) *Neck*

Cervical sclerites.—Smoky.

(d) *Thorax*

Pronotum.—Yellow with brighter specks, white along the anterior, ventral and posterior margins. Keel yellow in front and white behind the hind sulcus. Lateral black pattern absent, only faint smoky colour at the base of posterior sulcus. Smoky metazonal triangular patches with yellow dots on each side of the median line, narrower and darker in front, wider and fainter posteriorly.

Meso-episterna.—Yellow without any black dots.

Meso-epimera.—Uniformly yellow, whitish near the posterior margin.

Met-episterna.—Yellow, a black streak along the posterior margin.

Met-epimera.—Yellow, a black streak along the anterior margin.

Wing pads.—Black dots at the base of wings. Dots on dorsal longitudinal veins, lines on lower longitudinal veins.

Sterna.—Uniformly yellow.

(e) *Legs*

I and II.—Femur and tibia yellow, tarsi light brown ; claws and spines tipped with brown.

III.—Hind femur : on the anterior aspect black colour only on the dorsal 2/3 of the muscle-impression area, the remaining 1/3, above and along the antero-ventral femoral carina yellow. The black extensions at the distal end of muscle-impression area light, *i.e.*, smoky and enclose a wide patch of ground colour. These two extensions go up, cross the dorsal carina and end on the posterior aspect just below the postero-dorsal femoral carina. The distal arm is very faint on the posterior aspect.

(f) *Abdomen*

Segment I.—Tergum covered under wing-pads, uniformly yellow.

Segment II.—Dorsal black stripe on tergum absent. A row of four black dots dorso-ventrally on each lateral aspect.

Segments III-VIII.—On tergum, dorsal stripe blurred black, interrupted by a white patch at the posterior margin. Laterally five elongate black dots along the posterior margin. The ventralmost united with the straight black stripe, the 2nd opposite to a faint brown oblique streak usually cut up. Opposite to the 3rd and 4th are three or four dots irregularly arranged.

Segment IX.—Mid-dorsal stripe and lowermost straight stripe faint smoky, rest yellow.

Segment X
Telson } .—Yellow.

Ventral surface.—Yellow.

iv. Coloration of the V stage hoppers bred crowded at 44° C. (*Plate XL, fig. 5*)

General colour white, pale yellow tinge at some places. Eyes with three light brown stripes, interstripes white. Extremely faint, smoky sub-ocular genal stripe visible. Light brownish yellow along the posterior margin of the post-ocular region. Remaining face and antennae white.

Pronotum white behind the hind sulcus, pale yellow tinge in front of it.

Legs white, light brown fish-bone area on the anterior aspect of hind femur.

Abdomen white with a very light yellowish tinge. Faint smoky dots along the posterior margin of each abdominal segment.

IV. Concluding remarks on colour variations

From the detailed description of the hoppers of the different stages, bred crowded, at various constant temperatures, *viz.*, 24° C., 33° C., 40° C. and 44° C., it is possible to summarise the following general conclusions :—

(*a*) *General colour*

At 24° C. the black colour is most intense and the black pattern most extensive, *i.e.*, the black predominates. At 33° C. the pigmentation is generally reduced and the general body colour is light yellowish in II and III stages, and greenish or greenish yellow in IV and V stages. The black pattern is present at 40° C. although much reduced. At 44° C. the black pattern is almost absent. The colour is uniformly dull white to pale yellow and there are no black markings except faint smoky design in some cases. Thus with successive rise in temperature the black pigmentation gets reduced and is finally eliminated.

(*b*) *Head*

At 24° C. the entire front, clypeus, labium and genae arejet black, therefore the sub-ocular genal stripe is not distinguishable. At 33° C. the black colour is confined to the lower part of the head—the labrum is entirely black, while clypeus may be only black or spotted ; the frons has a uniform pinkish colour with fine black dots on the carinae. The black sub-ocular genal stripe is broad, creeping up along the margin of the compound eyes. The rest of the gena has the ground colour of the body. At 40° C. the entire face including frons, clypeus and labrum, has the uniform dull white colour in the II and III stages and yellow or greenish yellow in IV and V stages without any black markings or dots. The sub-ocular genal stripe is very narrow and smoky or has a bluish tinge. At 44° C. no black pigment is present.

Vertex and post-ocular region.—At 24° C. the epicranial suture is faint or indistinct and the vertex and post-ocular region are black, except a very reduced post-ocular brownish orange patch. At 33° C. there are well-marked triangular areas on each side of the epicranial suture. The lower angle of the triangles is continued downwards as a narrow streak behind the compound eyes joining the sub-ocular black portion. The post-ocular patch is extensive and orange-yellow in colour. At 40° C. broken triangular areas are present on each side of the epicranial suture and are not continued downward, nor fused with the sub-ocular genal stripe. The post-ocular patch is brownish in II stage and yellowish orange in the remaining stages. The rest of the post-ocular region has the ground colour of the body.

Compound eyes.—At 24° C. the eyes are not striped and have a uniform dark reddish brown coloration. At 33° C. the hoppers of II stage retain the uniform dark reddish brown coloration, but those of the later stages have striped eyes. The stripes are reddish brown with interstripes. There are three or four such stripes in III, four or five in IV and five or six stripes in V stage. At 40° C. the eyes are striped in all the stages. In II stage the anterior half is light grey and the posterior is dark brown. This fact is of interest because the striped eyes are regarded as a character of the *solitary* hopper. In these experiments the crowded hoppers have attained the striped conditions.

The situation may be explained thus. The eyes are essentially striped in *Schistocerca gregaria*, but these stripes are concealed by superimposed pigmentation and when that is removed through any cause, the stripes become evident.

Antennae.—At 24° C. and 33° C. the antennae are black excepting the basal two segments which are lighter, and a lighter line along the lateral aspect of the antennae. At 40° C. the antennae are smoky in the middle and whitish at the base and tip.

(*c*) *Cervex*

The cervical sclerites are black at 24° C. and 33° C., but lighter, *i.e.*, smoky at 40° C.

(*d*) *Thorax*

Pronotum.—At 24° C. in II, III and IV instars there are prominent black stripes on each side of the median line, which are usually fused with each other in the prozonal region. They widen up anteriorly to form small black triangular areas, and also posteriorly to form triangular metazonal patches. In the lateral region there is a large black pattern covering the whole of prozona and greater portion of the metazona, leaving only a comma-shaped patch of ground colour. There are usually whitish or yellowish white dots on the lateral black pattern in III and IV stages. In V stage the entire pronotum may become black with numerous yellowish dots.

At 33° C. the mid-dorsal line on the pronotum is white, with a narrow black stripe on its either side which widens up posteriorly forming the usual black triangular metazonal patches. The lateral black pattern is less extensive, usually lighter in the prozonal region and leaving a broader comma-shaped patch of basic colour along the dorsal and posterior margin of pronotum.

At 40° C. there are narrow smoky stripes on each side of a whitish median line with the smoky triangular metazonal patches at their posterior end. The lateral black pattern is almost absent in the prozonal region and only faint smoky marks may be present in the metazonal region. At 44° C. no black pigment is left.

Meso- and meta-nota.—In II and III stages the meso and meta-nota carry a mid-dorsal black stripe in continuation of the black stripe on pronotum. Below this on each side is a whitish stripe in continuation of the similar stripe on pronotum. The remaining area below this white stripe is black.

At 24° C. and 33° C. the mid-dorsal black stripes are wider and more intense leaving a relatively narrow lateral white stripe. At 40° C. the stripes are very narrow and smoky and at 44° C. the colour is dull white.

In IV and V stages the meso and meta-notum are covered under wing pads and do not show the pattern found in the II and III stages.

Lateral thoracic sclerites.—The pro-episternum is black at 24° C. and dull white to yellowish at higher temperatures.

At 24° C. the meso-episternum and meso-epimeron have the basic colour of the body with smoky or black patches at different places.

The met-episternum and met-epimeron are uniformly black at 24° C. At 33° C. the met-episternum is mixed black and white, while met-epimeron remains uniformly black. At 40° C. the met-episternum and met-epimeron are uniformly white or yellow, with usually black marks along the sutures.

Sternum.—At 24° C. the ventral side of thorax has numerous dark brown to black patches ; at 33° C. there are smoky dots on the basic coloration, while at 40° C. the ventral thorax is almost uniformly white or yellow.

(*e*) *Legs*

I and II pairs.—At 24° C. and 33° C. the first two pairs of legs have numerous smoky dots with extensive black rings along the head and apex of femur and tibia. The spines are black and the tarsi, dark brownish.

At 40° C. the dots are faint brownish and only smoky spots are present on the head and apex of femur and tibia. The tarsi are lighter at 44° C.

Hind legs.—At 24° C. the anterior muscle-impression area of femur is completely black, and pigmentation is extensive. The black patterns on the anterior and posterior surfaces are almost fused dorsally with the exception of a narrow oval gap near the distal end.

At 33° C. the black pattern on the anterior muscle-impression area is less broad and leaves a narrow streak of basic colour above the antero-ventral femoral carina. It, however, possesses two black dorsal extensions near the distal end which enclose a gap of basic colour and usually end on the postero-ventral femoral carina.

At 40° C. the black pattern is narrow and covers only upper 2/3 of the anterior muscle-impression area, the remaining 1/3 along the antero-ventral femoral carina is yellowish. The two dorsal extensions at the distal end are narrow and broken and enclose a relatively larger oval gap. They end at the postero-dorsal femoral carina.

In the hoppers of the II stage at 24° C. and 33° C. the posterior muscle-impression area is also black, like the anterior one.

Markings on the meta-femur, meta-tibia and meta-tarsi undergo changes almost like those in the I and II pairs of legs. The distance between the posterior end of the femoral black pattern and the black ring at the apex of femur increases as the temperature rises.

At 44° C. no black pigment is left.

(*f*) *Abdomen*

Segment I.—In II and III stage hoppers there being no wing pads, segment I is not covered. The mid-dorsal stripe is always present with the ivory coloured triangle at the posterior margin, but the black stripe is broader and intenser at 24° C. and narrower and divided into two by a fine white median line at 33° C. and 40° C. Laterally there are a number of black dots along the posterior margin and above the ear, but their number and intensity of colour decreases as the temperature rises from 24° C. to 40° C.

In IV stage hoppers, segment I is partly covered under the wing pad and shows partial black markings. At 24° C. the mid-dorsal tergal stripe is broad and black and with the usual mid-dorsal ivory patch at the posterior margin of each segment. Laterally there are a few black dots along the posterior margin with a few more in front of it. At 33° C. the mid-dorsal stripe is faint and laterally there are only four or five dots along the posterior margin, while at 40° C. and 44° C. the black markings are absent. A few faint smoky dots may be present in the lateral aspect.

In the hoppers of V stage this segment is completely covered under the wing pads so that there are hardly any markings on it.

Segments II-VIII.—At 24° C. the dorsal black stripe is broad and undivided, with the usual white triangular area at the posterior end. Laterally above the white spiracular stripe, there is a broad straight black stripe with its anterior end much wider. Above this, is a black oblique streak and a number of black dots arranged in a row parallel to the oblique streak. Lastly there are two or three dots above this which are sometimes fused into black spots. Very little of the ground colour of the body is visible laterally. The same pattern is maintained at 33° C. and 40° C. but the streaks as well as dots become lighter and narrower.

Segments IX-X.—At 24° C. the 9th and 10th segments are almost entirely black. At higher temperatures, the mid-dorsal stripe is present in continuation of previous segments but it is narrower. The lateral aspect does not have the usual pattern consisting of regular streaks, but possesses a row or two of dots only.

Telson.—At 24° C. the telson is brown to black ; at higher temperatures it has the basic colour of the body with or without smoky spots.

Ventral surface of abdomen.—At 24° C. the ventral surface of abdomen is uniformly brown to black, excepting a lighter mid-ventral line running along the length of abdomen. At 33° C. the ventral surface is dull white with many smoky or brownish dots. At 40° C. the entire ventral surface is uniformly white or yellow.

NOTE.—The coloration of hoppers at 24° C. is the nearest approach to that of the hoppers met with normally in a swarm of spring brood, while the coloration of hoppers at 33° C. represents very nearly that of the hoppers of the summer brood.

V. Discussion

Several theories have been advanced to explain different types of colour forms among insects. Standfuss [1896] and Hudson [1913] regarded the development of pigmentation as a phenomenon of adaptation, the dark coloured forms in humid places, and near industrial towns, appearing in response to their more or less dark-coloured surroundings, providing protective coloration for the organism. The development of pigmentation in the '*gregarious*' hoppers cannot be explained on the basis of protective or concealing coloration. The '*solitary*' hoppers with their general light green colour are very well-concealed among green bushes, while the '*gregarious*' hoppers with their black and yellow colour are most conspicuous on the desert sand on which they venture out, 'without cover', in thick swarms.

Knight [1924] at first regarded the white and pigmented forms of *Perillus bioculatus* as two independent forms, but after a large number of breeding experiments he was not able to establish pure lines of non-pigmented and pigmented forms. He was, therefore, forced to the conclusion that the colour patterns of individual bugs were influenced by external conditions and were not characters inherited from their parents. This is exactly the situation with respect to the Desert Locust. External conditions determine the coloration of the hoppers, irrespective of the coloration of the parents and irrespective of the coloration, which an hopper possesses in the first stage. The most important of these external factors is, of course, the density of population in which the hoppers are bred, whether under natural conditions or in a cage. This correlation of the intensity of population and coloration greatly complicates the question of pigmentation in locusts.

Temperature also influences coloration. The production of dark colour aberrations in *Leptinotarsa* sp. at both high and very low temperatures has been explained by the failure of the action of ferments which are adversely affected at both these limits [Przibram, 1919].

Linden [1904] explains the colour variation 'of temperature forms' on the basis of changes in metabolism and particularly in the rate of respiration. According to Prochnow [1929] one of the functions of pigment-formation is the disposal of waste products. The waste products, which cannot be excreted from the body, are deposited as pigment. From this it follows that greater the rate of metabolism, greater the quantity of waste products and higher the deposition of pigment. Schulze [1917] maintains that since the males show a more active oxidation than the females, we must have more strongly black-pigmented forms among the males than among the females. On the basis of these theories one ought to get black pigments intensifying at high temperature, but this does not happen in *Schistocerca gregaria.*

Faure [1932] investigating the causes underlying the developments of the *gregaria* black coloration in locusts proposes the theory of *locustine* production. As a result of the excessively high rate of metabolism obtaining in swarm life *locustine* is produced, and this leads to the development of black pigmentation. According to this theory increased metabolism as a result of great muscular activity at high temperature must produce larger amount of *locustine* and consequently more darkly pigmented forms, which is not the case in *Schistocerca gregaria.*

Contrary to these views, Knight [1924], basing his opinion on his experiments on *Perillus bioculatus*, maintains that increased metabolism produces de-pigmented forms. He argues that since the females have to mature large number of eggs, the metabolism in this sex goes on at a higher rate, than in the male, consequently the females are whitish between 78-82° F., while males are coloured. The physiological activity of these bugs can also be raised by rearing them at a temperature of 85° F. or higher, when the white forms appear. He maintains that restricted movements, in other words, less exercise, also gives darker forms. Therefore according to this investigator increased metabolism does not produce pigment, but on the contrary, consumes or otherwise disposes of the materials, which under low temperature are laid down as pigment in the body wall. This view is also supported by the studies of Genieys [1925], Schlottke [1926], and Kühn and Henke [1929].

According to Schroder [1903], dark pigments are formed under the influence of cold so as to increase the absorption of heat by the insect and thus counterbalance the influence of cold on development. This theory does not explain the origin of pigmentation ; it only explains the consequence of the development of pigmentation.

The influence of temperature on the pigmentation of Desert Locust hoppers bred crowded seems to be exactly like that mentioned by Knight. More deeply pigmented forms are produced at low temperature, while less pigmented forms result from breeding at high temperature. In regard to the influence of muscular activity, however, the results of experiments conducted [Afzal Husain and Mathur, 1936] are very different. Increased muscular activity in their experiments produced darker forms.

These studies thus show that whatever the significance of the development of black pigmentation, the causes leading to it are physiological and not morphological. And since the effect of temperature on physiological processes is different in different groups of insects it would not be safe to make generalization, each group of insects meriting special study.

In the Desert Locust, as in some other locusts, the *swarming* phase differs from the *solitary* phase, in addition to coloration, in some anatomical details, expressed in differences in the shape and size of certain portions of the body. It will be worth investigating if coloration and anatomical differences go together. This problem will receive attention in a subsequent paper. It appears evident that one cannot place too much reliance on coloration in distinguishing phases and sub-phases.

VI. Summary

The temperature at which locust hoppers are bred has a marked influence on the intensity as well as the extent of pigmentation in the hoppers of *Schistocerca gregaria* bred crowded.

Hoppers bred crowded at 24° C. develop deep and extensive black patterns on the various parts of the body and the black colour is predominent.

At high temperature the black markings get reduced and the basic yellow or greenish yellow colour predominates.

At a still higher temperature of 44° C. the black markings are almost entirely absent, and the hoppers are whitish.

The present studies indicate that the causes leading to pigmentation are physiological and not morphological. The influence of temperature on pigmentation would therefore be determined by the manner in which the physiological processes are affected in different groups of insects.

These experiments also show that all stages in the variation in colour from black to white can be produced in the crowded hoppers under the influence of temperature. Incidently one may ask if reliance can be placed on colour in making distinction between the different phases and sub-phases.

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TABLE I

Life-history of hoppers reared at different constant temperatures . Experiment I
Reference No. L 224

Date	Days	24° C.	24° C.	27° C.	30° C.	30° C.	33° C.	33° C.	36° C.	36° C.	36° C.	40° C.
11th Nov. 1932 . .	0	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)
12th ,, . .	1											
13th ,, . .	2											
14th ,, . .	3									II (3)		II (3)
15th ,, . .	4						II (3)	II (3)	II (3)		II (3)	
16th ,, . .	5				II (3)	II (3)						D (1)
17th ,, . .	6			II (3)								III (2)
18th ,, . .	7				D (1)				III (3)	III (3)	III (3)	
19th ,, . .	8	II (3)					III (3)	III (3)				
20th ,, . .	9		II (3)									
21st ,, . .	10		D (1)		III (2)	III (3)						IV (2)
22nd ,, . .	11			D (1)					IV (3)	IV(3)	IV (3)	
23rd ,, . .	12			III (2)			IV (3)	IV (3)				
24th ,, . .	13											
25th ,, . .	14					D (1)					D (1)	V (2)
26th ,, . .	15				IV (2)	IV (2)			V (3)	V (3)	V (2)	

TABLE I—*contd.*

Date	Days	24° C.	24° C.	27° C.	30° C.	30° C.	33° C.	33° C.	36° C.	36° C.	36° C.	40° C.
27th Nov. 1932 .	16	III (3)	III (2)							D (1)		
28th ,, . .	17						V (3)	V (3)				
29th ,, . .	18											
30th ,, . .	19			IV (2)								
1st Dec. 1932 .	20				V (2)	V (2)						
2nd ,, . .	21											A (2)
3rd ,, . .	22								A (3)	A (2)	A (2)	
4th ,, . .	23											
5th ,, . .	24							D (1)				
6th ,, . .	25											
7th ,, . .	26	IV (3)					A (3)	A (2)				
8th ,, . .	27		IV (2)	D (1)								
9th ,, . .	28			V (1)								
10th ,, . .	29											
11th ,, . .	30											
12th ,, . .	31				A (2)	A (2)						
13th ,, . .	32											
14th ,, . .	33											

15th ,, . .	34											
16th ,, .	35											
17th ,, . .	36											
18th ,, . .	37											
19th ,, . .	38											
20th ,, . .	39	V (3)										
21st ,, . .	40		V (2)									
22nd ,, . .	41											
23rd ,, . .	42											
24th ,, . .	43											
25th ,, . .	44											
26th ,, . .	45											
27th ,, . .	46											
28th ,, . .	47			A (1)								
29th ,, . .	48											
30th ,, . .	49											
31st ,, . .	50											
1st Jan. 1933 .	51											
2nd ,, . .	52											
3rd ,, . .	53	D (1)										
4th ,, . .	54											
5th ,, . .	55											
6th ,, . .	56											

TABLE I—*concld.*

Date	Days	24° C.	24° C.	27° C.	30° C.	30° C.	33° C.	33° C.	36° C.	36° C.	36° C.	40° C.
7th Jan. 1933 .	57											
8th ,, . .	58											
9th ,, . .	59	D (1)										
10th ,, . .	60											
11th ,, . .	61											
12th ,, . .	62	A (1)	A (2)									
Duration of hopper stage in days		62	62	47	31	31	26	26	22	22	22	21

Roman numbers represent instars of the hoppers. Arabic numbers in ( ) represent the number of hoppers in the cage. D=Died and figure in ( ) represents number dead. A=Adult, and figure in ( ) represents their number.

TABLE II

Life-history of hoppers reared at different constant temperatures. Experiment II
Reference No. P 74

Date	Days	24° C.	27° C.	30° C.	33° C.	36° C.	40° C.
14th Jan. 1933	0	I (3)	I (3)	I (3)	I (3)	I (3)	I (3)
15th ,,	1						
16th ,,	2	D (1)					
17th ,,	3			D (1)		II (3)	II (3)
18th ,,	4			II (2)	II (3)		
19th ,,	5						
20th ,,	6		II (3)			III (3)	III (3)
21st ,,	7						
22nd ,,	8	II (2)			III (3)		
23rd ,,	9			III (2)			D (1)
24th ,,	10					IV (3)	IV (2)
25th ,,	11						
26th ,,	12		III (3)		IV (3)		
27th ,,	13						
28th ,,	14			IV (2)		V (3)	V (2)
29th ,,	15						
30th ,,	16	III (2)					
31st ,,	17				V (3)		
1st Feb. 1933	18		D (1)				
2nd ,,	19		IV (2)				
3rd ,,	20			V (2)			
4th ,,	21					A (3)	A (2)
5th ,,	22						

TABLE II—*contd.*

Date	Days	24° C.	27° C.	30° C.	33° C.	36° C.	40° C.
6th Feb. 1933	23						
7th ,,	24						
8th ,,	25						
9th ,,	26				A (3)		
10th ,,	27	IV (2)	V (2)				
11th ,,	28						
12th ,,	29						
13th ,,	30						
14th ,,	31			A (2)			
15th ,,	32						
16th ,,	33						
17th ,,	34						
18th ,,	35						
19th ,,	36						
20th ,,	37						
21st ,,	38						
22nd ,,	39	D (1)					
23rd ,,	40	V (1)					
24th ,,	41						
25th ,,	42						
26th ,,	43						
27th ,,	44						
28th ,,	45						
1st Mar. 1933	46		A (2)				
2nd ,,	47						
3rd ,,	48						

TABLE II—*concld.*

Date	Days	24° C.	27° C.	30° C.	33° C.	36° C.	40° C
4th Mar. 1933 .	49						
5th	50						
6th ,, . . .	51						
7th ,, . . .	52						
8th ,, . . .	53						
9th ,, . . .	54						
10th ,, . . .	55						
11th ,, . . .	56						
12th ,, . . .	57						
13th ,, . . .	58						
14th ,, . . .	59						
15th ,, . . .	60						
16th ,, . . .	61						
17th ,, . . .	62						
18th ,, . . .	63	A (1)					
Duration of hopper stage (days).		63	46	31	26	21	21

TABLE III.

Life history of hoppers reared at different constant temperatures Experiment III Reference No. L 276

Date	Days	24° C.	27° C.	30° C.	33° C.	36° C.	40° C.
16th Jan. 1933 .	0	I (7)	I (7)	I (7)	I (7)	I (7)	I (7)
17th ,, . . .	1	D (1)	D (2)				D (1)
18th ,, . . .	2						
19th ,, . . .	3					II (7)	II (6)

TABLE III—*contd.*

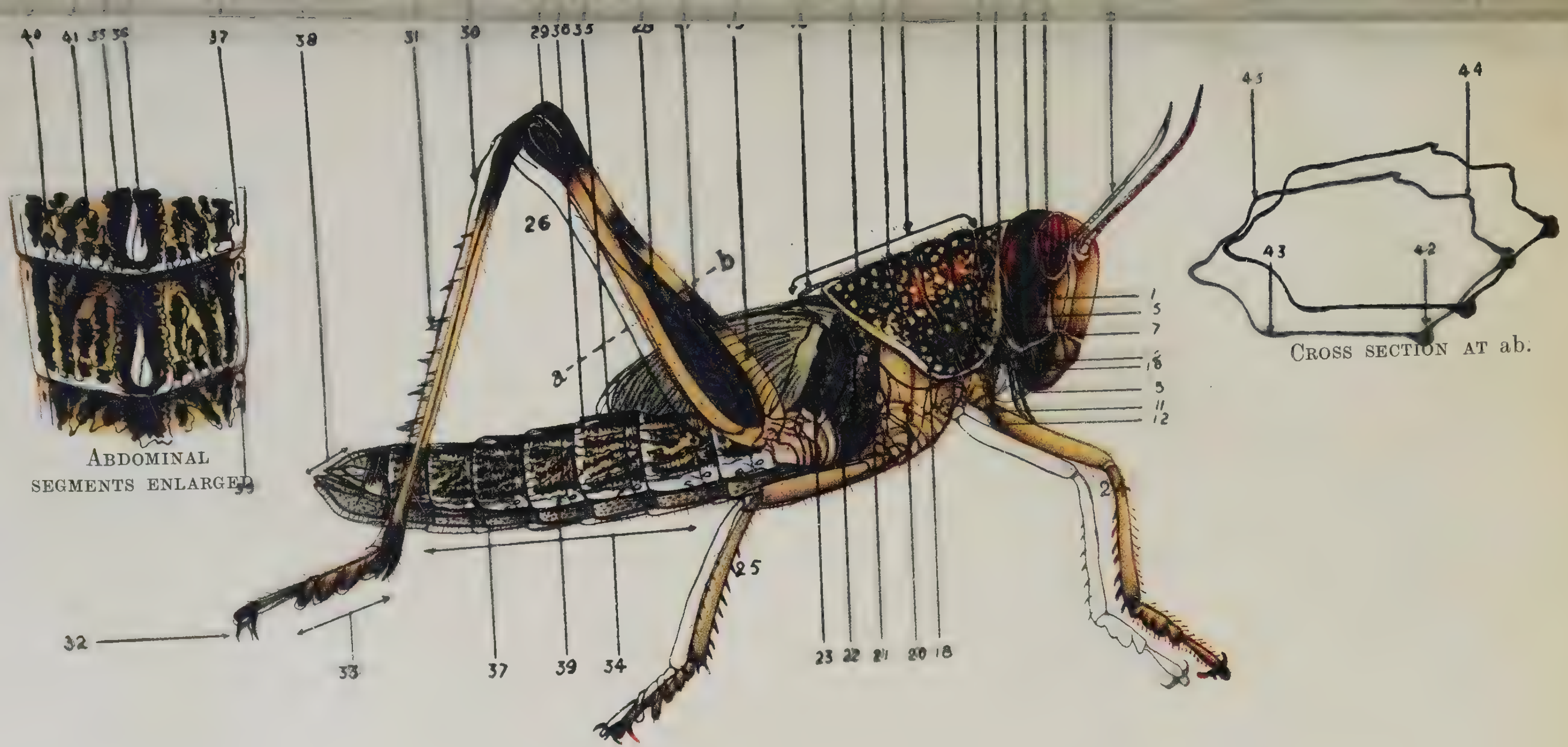
Date	Days	24° C.	27° C.	30° C.	33° C	36° C.	40° C
20th Jan. 1933 .	4				II (7)		
21st ,, . . .	5			II (7)			
22nd ,, . . .	6		II (5)			III (7)	III (6)
23rd ,, . . .	7						
24th ,, . . .	8	II (6)			D (2)		
25th ,, . . .	9	D (2)		III (7)	III (5)		
26th ,, . . .	10					IV (7)	IV (6)
27th ,, . . .	11						
28th ,, . . .	12		D (1)			D (1)	
29th ,, . . .	13		III (4)		IV (5)	D (1)	D (1)
30th ,, . . .	14			IV (7)		V (5)	V (5)
31st ,, . . .	15						
1st Feb. 1933 . .	16						
2nd ,, . . .	17	III (4)					
3rd ,, . . .	18				V (5)		
4th ,, . . .	19						D (1)
5th ,, . . .	20		IV (4)	V (7)			
6th ,, . . .	21					A (5)	A (4)
7th ,, . . .	22						
8th ,, . . .	23						
9th ,, . . .	24			D (1)			
10th ,, . . .	25						
11th ,, . . .	26						
12th ,, . . .	27	D (1)			A (5)		
13th ,, . . .	28	IV (3)					
14th ,, . . .	29		V (4)				

TABLE III—*contd.*

Date	Days	24° C.	27° C.	30° C.	33° C.	36° C.	40° C.
15th Feb. 1933 . .	30						
16th " . . .	31			A (6)			
17th " . . .	32						
18th " . . .	33						
19th " . . .	34						
20th " . . .	35						
21st " . . .	36						
22nd " . . .	37						
23rd " . . .	38		D (1)				
24th " . . .	39						
25th " . . .	40						
26th " . . .	41	V (2)					
27th " . . .	42						
28th " . . .	43						
1st Mar. 1933 . .	44						
2nd " . . .	45						
3rd " . . .	46						
4th " . . .	47		A (3)				
5th " . . .	48						
6th " . . .	49						
7th " . . .	50						
8th " . . .	51						
9th " . . .	52						
10th " . . .	53						
11th " . . .	54						
12th " . . .	55						

TABLE III—*concld.*

Date	Days	24° C.	27° C.	30° C.	33° C.	36° C.	40° C.
13th Mar. 1933 . .	56						
14th ,, . . .	57						
15th ,, . . .	58						
16th ,, . . .	59						
17th ,, . . .	60						
18th ,, . . .	61						
19th ,, . . .	62						
20th ,, . . .	63						
21st ,, . . .	64	A (2)					
Duration of hopper stage in days.		64	47	31	27	21	21


40
41
35
36
37
38
31
30
29
30
35
26
b
a
1
5
7
18
5
11
12
2
25
32
33
37
39
34
23
22
21
20
18
45
44
43
42
ABDOMINAL SEGMENTS ENLARGED
CROSS SECTION AT ab.

1. Frons.
2. Clypeus.
3. Labrum.
4. Vertex.
5. Gena.
6. Subocular genal stripe.
7. Post gena.
8. Compound eye.
9. Antenna.
10. Mandible.
11. Maxillary palp.
12. Labial palp.
13. Post-ocular patch.

16. Keel
17. Metazonal triangular patch.
18. Lateral black patch.
19. Wing pad.
20. Meso-episternum.
21. Meso-epimeron.
22. Met-episternum.
23. Met-epimeron.
24. Leg, I pair.
25. Leg, II pair.
26. Leg, III pair.
27. Hind femur.
28. Anterior muscle impression.
29. Apex of femur.

31. Tibial spines.
32. Claws.
33. Tarsi.
34. Abdomen.
35. Mid-dorsal black stripe.
36. White mid-dorsal triangular area.
37. Lateral spiracular stripe.
38. Telson and Cerci.
39. Spiracle.
40. Lateral straight stripe.
41. Lateral oblique streak.
42. Antro-dorsal carina.
43. Antro-ventral carina.
44. Postero-dorsal carina.

GREGARIOUS HOPPERS BRED CROWDED AT A TEMPERATURE OF 24°C.

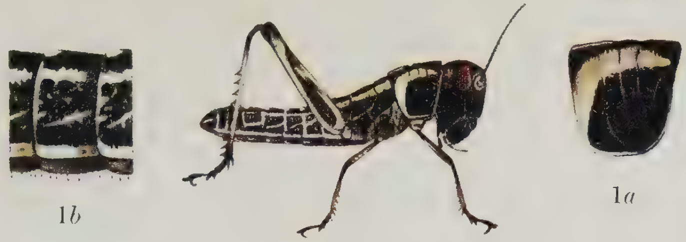


FIG. 1. Hopper of the second stage (×2·2).

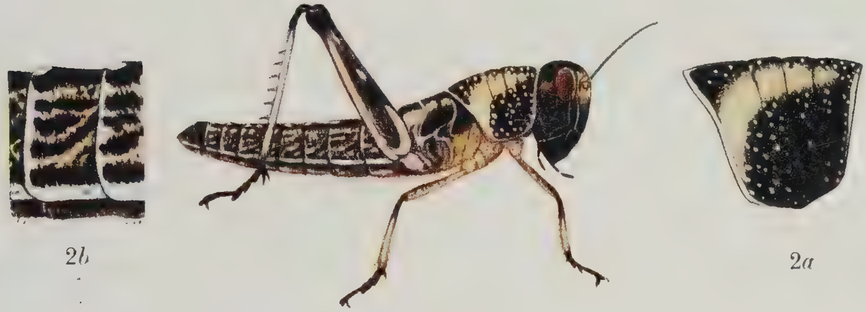


FIG. 2. Hopper of the third stage (×1·8).

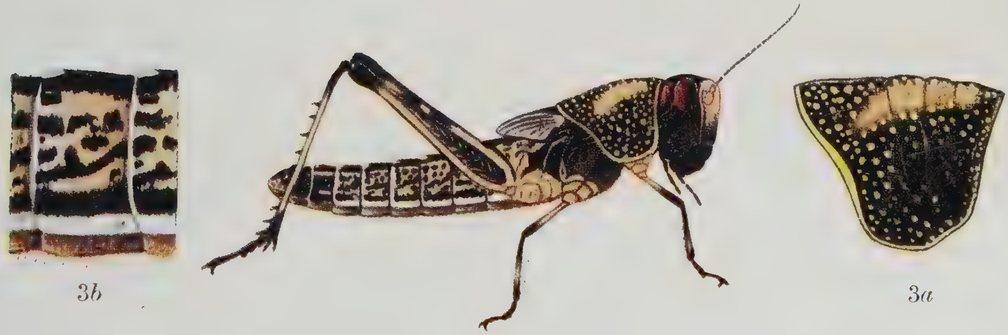


FIG. 3. Hopper of the fourth stage (×1·5).

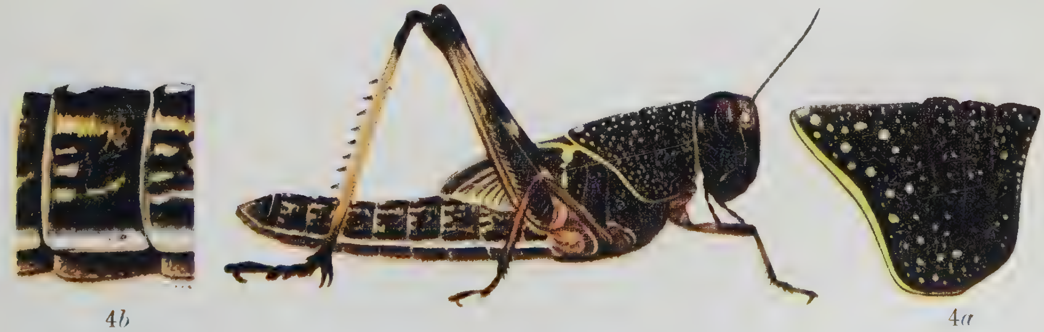


FIG. 4. Hopper of the fifth stage (×1·1).

a. Pronotum enlarged, *b*. Abdominal segments enlarged.

GREGARIOUS HOPPERS BRED CROWDED AT A TEMPERATURE OF 33°C.

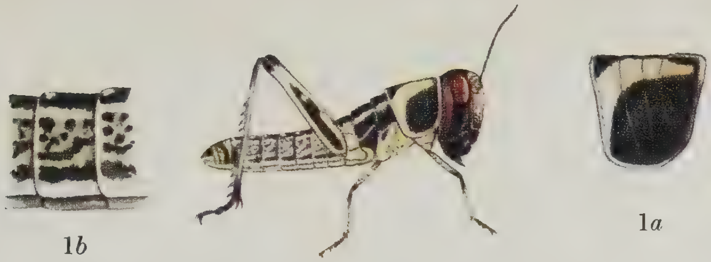


FIG. 1. Hopper of the second stage (×2·2).

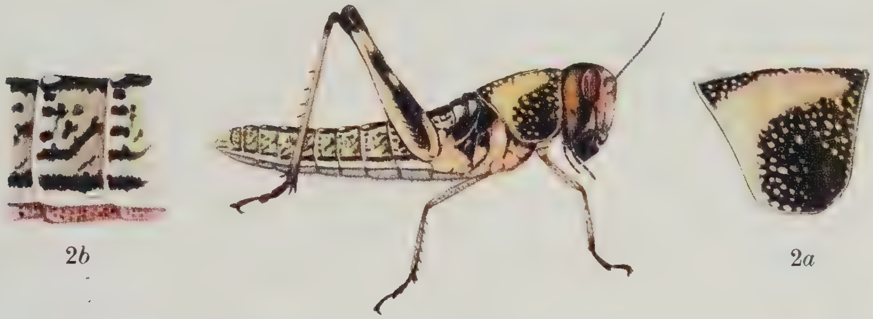


FIG. 2. Hopper of the third stage (×1·8).

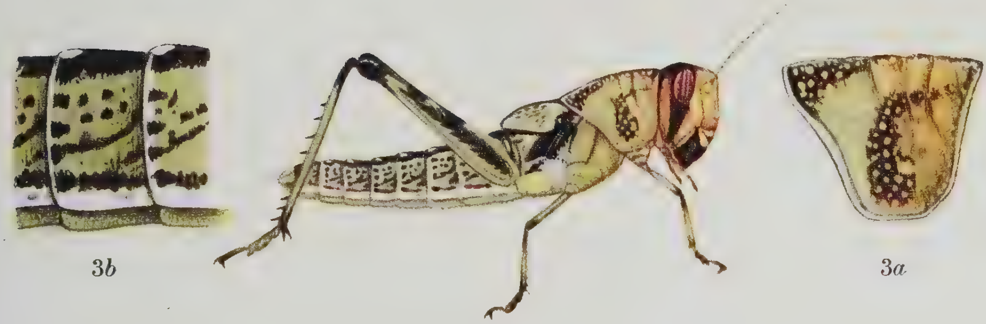


FIG. 3. Hopper of the fourth stage (×1·5).

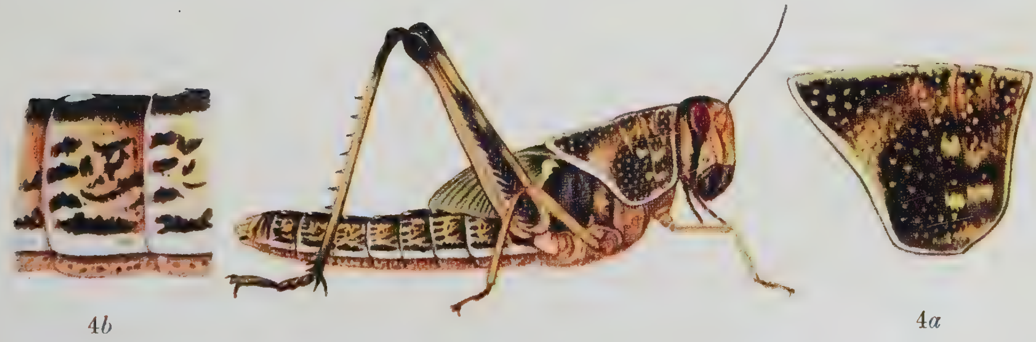


FIG. 4. Hopper of the fifth stage (×1·1).

a. Pronotum enlarged, *b*. Abdominal segments enlarged.

GREGARIOUS HOPPERS BRED CROWDED AT A TEMPERATURE OF 40°C.

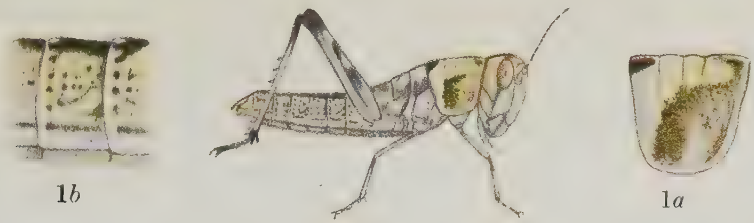


Fig. 1. Hopper of the second stage (×2·2).

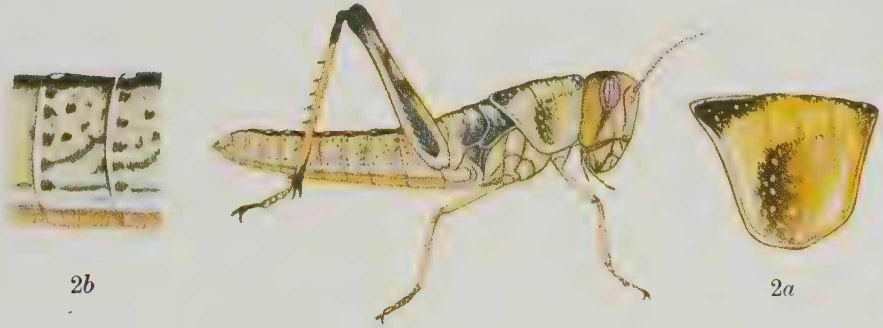


Fig. 2. Hopper of the third stage (×1·8).

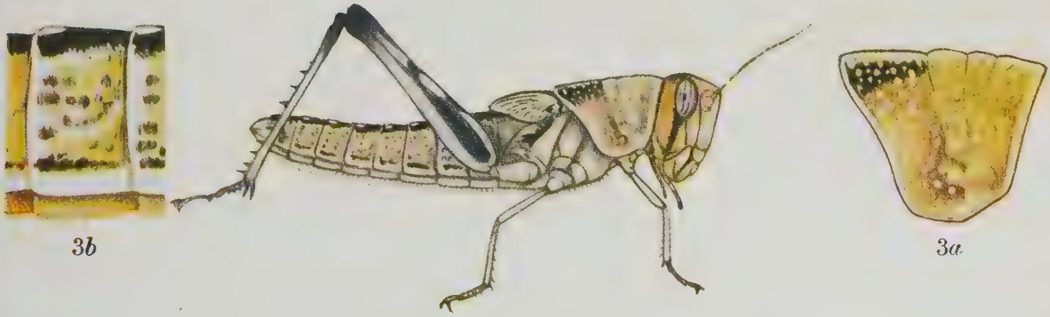


Fig. 3. Hopper of the fourth stage (×1·5).

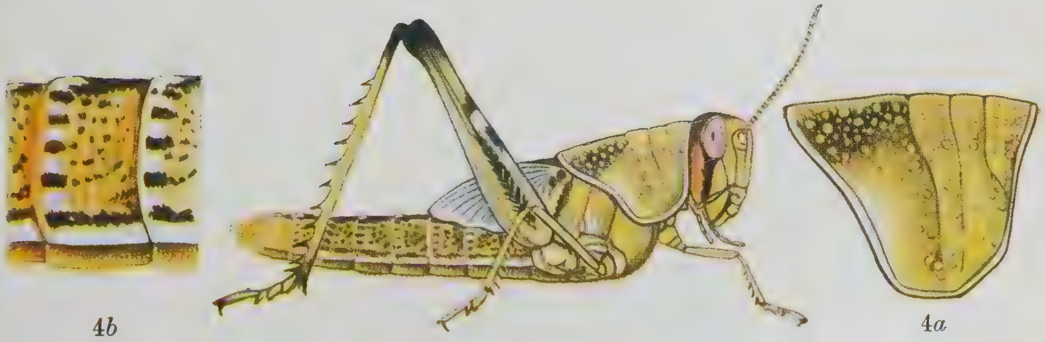


Fig. 4. Hopper of the fifth stage (×1·1).

(*a*) Pronotum enlarged, (*b*) Abdominal segments enlarged.

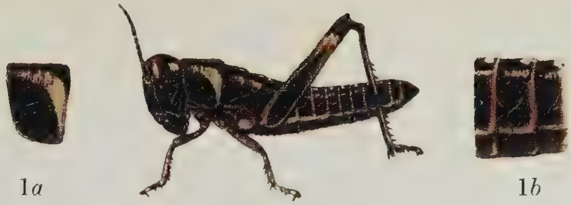


FIG. 1. Hopper of the first stage, which was the starting point of experiment (×2·8)

(*a*) Pronotum enlarged (*b*) Abdominal segments enlarged.

HOPPERS BRED CROWDED AT 44° C.

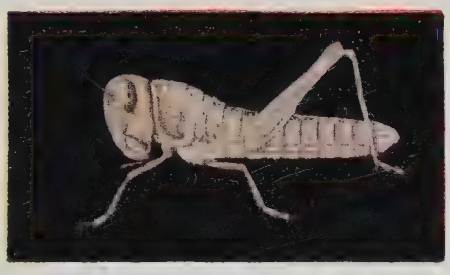


FIG. 2. Hopper of the second stage (×2·8)

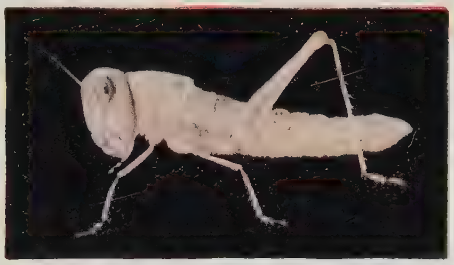


FIG. 3. Hopper of the third stage (×1·7)

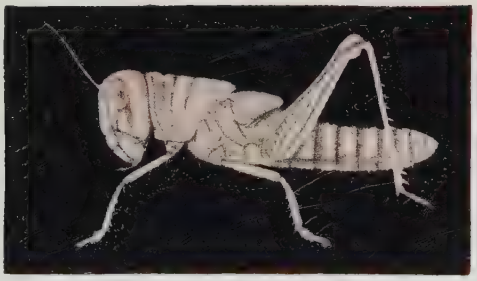


FIG. 4. Hopper of the fourth stage (×1·7)

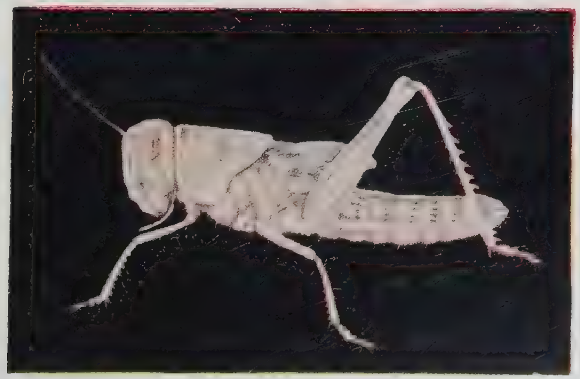


FIG. 5. Hopper of the fifth stage (×1·2)

STUDIES ON *SCHISTOCERCA GREGARIA* FORSK.

*VII. FACTORS DETERMINING THE MOVEMENT OF THE VERMIFORM LARVAE

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(Received for publication on 6th January 1936)

(With four text-figures)

INTRODUCTORY

Like most other Locustidae (Acridiidae), *Schistocerca gregaria* Forsk. normally lays its eggs in holes made in the soil. These burrows ordinarily are about 8-13 cm. deep. The eggs are deposited in an elongated pod at the blind end of this burrow. While the eggs are being passed out of the body of the female, a viscous matter is also poured out, and with the air bubbles present, it forms a foamy material which sets as a frothy or spongy covering of the egg-pod. After all the eggs have been laid the female gradually withdraws its abdomen from the burrow and while doing so she pours out a mass of this foamy material which sets into a frothy or spongy plug or stopper, which occupies the entire space in the burrow not occupied by the egg-pod. The eggs thus lie a few centimeters below the soil surface, in a

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*Studies on *Schistocerca gregaria* Forsk.

I. The Micropyle in *Schistocerca gregaria* Forsk. and some other Acridiidae. *Ind. J. Agric. Sci.* **3** : 639-645.

II. The Biology of the Desert Locust with special relation to Temperature. *Ind. J. Agric. Sci.* **6** : 188-262.

III. Why do Locusts Eat Wool ? A Study in the Hydromania of *Schistocerca gregaria*. *Ind. J. Agric. Sci.* **6** : 263-67.

IV. Colour Changes and Sexual Behaviour in Desexualised *Schistocerca gregaria* Adults. *Ind. J. Agric. Sci.* **6** : 586-90.

V. Pigmentation and Physical Exertion. *Ind. J. Agric. Sci.* **6** : 591-623.

VI. Influence of Temperature on the Intensity and Extent of Black Pattern in the Desert Locust Hoppers bred Crowded. *Ind. J. Agric. Sci.* **6** : 624-664.

burrow closed with a spongy plug. This spongy plug may sometimes come slightly above the surface of the soil, but generally it stops a little below the upper opening of the burrow. Very often the mouth of the burrow gets closed by the disturbance of the soil.

From what has been said above, it is evident that the larvae hatching a few centimeters below the surface of the soil have to work their way out.

As is well known, the insect as it comes out of the egg shell or chorion is not a hopper but a vermiform larva. In other words the future hopper is enclosed in a thin membraneous overall, and its appendages lie in small blind sleeves of this overall, and are not capable of free movement. The only method of progression, which this vermiform larva is capable of, is wriggling like a worm ; hence the name vermiform larva. These larvae thus wriggle their way out through the frothy plug and the soil above and reach the surface where the 'intermediate' moult occurs and the larval skin is shed and the first instar hoppers emerge.

The question arises : What directs the vermiform larvae towards the surface of the soil ? The present state of our knowledge regarding this interesting phenomenon has been admirably summarised by Uvarov [1928] in the following :—

" The causes that direct the movement of the emerging larvae upwards have never been studied, although the phenomenon is of considerable interest. Theoretically it may be suggested that phototropism of the larvae plays a certain role, though on the other hand, larvae hatching in a glass jar containing earth move upwards along its transparent walls as usual. Apparently the movement is influenced by gravity (negative geotropism) and this suggestion is supported by the fact that the larvae that emerge from an egg turned upside down also move upwards ."

Thus two forces have been credited as providing the stimulus—positive phototropism and negative geotropism. Our experiments, however, show that an absolutely different factor is responsible, for directing the movement of the larvae out of the soil.

POSITIVE PHOTOTROPISM

The fact, that the vermiform larvae in the soil, particularly when the opening of the burrow is closed, cannot be directed by light, is in itself a definite proof that phototropism plays no role in this directive movement. During our investigation numerous cages were kept in incubators placed in a photographic dark room, and it was found that the emergence of the vermiform larvae from the soil was in every case quite normal.

NEGATIVE GEOTROPISM

Definite experiments have been performed to determine if geotropism is the directive factor, but the results obtained show that it is not so.

Experiment 1.—A fertilized female was caged in a circular glass dish 15 cm. in diameter and 9·5 cm. deep (Fig. 1). At the bottom of the dish a layer of moist soil 3·9 cm. in thickness was placed. On 23rd February 1931 the female had bored into the soil almost vertically and having come against the bottom of the dish she continued to bore parallel to the bottom. She thus made a vertical burrow 3·9 cm. deep and a horizontal burrow 8·6 cm. long, along the bottom of the dish. Eggs were laid at the blind end of this burrow. The egg-pod which was 5·1 cm. long was parallel to the bottom of the dish and consequently the long axis of the eggs was horizontal.

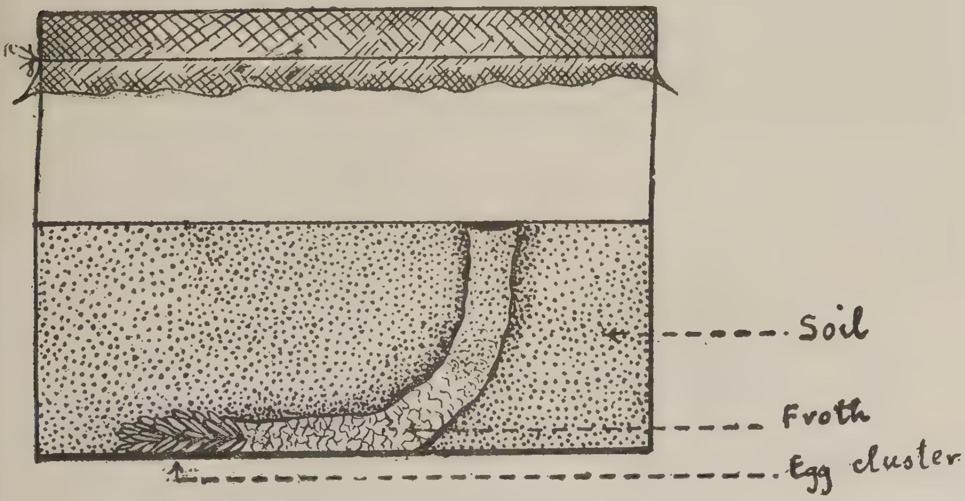

Fig. 1.

The remainder of the horizontal limb of the burrow was closed by the plug of froth 3·5 cm. in length. This plug reached the end of the horizontal portion of the burrow. The vertical part of the burrow got closed on account of the soili n the dish being moistened with water from time to time.

At 12-10 hours on 20th March 1931 when the dish was examined the eggs were hatching. The movements of the larvae during their attempt to come out of the burrow were studied. At the time of observation six larvae were seen in a line near the junction of vertical and the horizontal limbs of the burrow, trying to force their way out. Failing in their attempt to make a path through the soil, at about 1-45 P. M. the larvae turned back towards the blind end of the burrow where the egg-pod lay. By this time more of the larvae had hatched out and had reached the froth. Thus the larvae in front being unable to go back, moved in a circle at the end of the horizontal limb of the burrow. After about two and a half hours the dish was placed on edge in such a manner that the egg-pod was in a vertical position with the plug of forth, downwards. The larvae did not make any attempt to go up towards the egg-pod. They did not show any response to go against the force of gravity but continued to move in a circle and those behind continued to move down in the plug of froth. In other words the movements were quite independent of the directive force of gravity.

As the soil was rather hard for the larvae to make their way through, a hole was made for them to come out. The vermiform larvae now came out of this hole and shed their skin.

Experiment 2.—A fertilized female was made to lay eggs in an ordinary glass test-tube filled with soil. The egg-pod was visible through the walls of the tube. The length of the egg-pod was 2·5 cm. The plug of froth which extended to the surface of the soil was 6 cm. of which the lower 4 cm. could be seen clearly. The closed bottom end of this tube was broken. The length of the column of sand between this end and the bottom of the egg-pod was 6·4 cm. This tube containing the egg-pod was placed upside down, over a glass chimney so that the egg-pod was still held in a vertical position but its normal lower end was above, and the plug of froth below (Fig. 2). Except this the egg-pod was not disturbed in any way.

The eggs hatched on 14th April 1931 and all the fifty-two larvae worked their way downwards through the plug of froth and dropped down at the bottom of the glass chimney, the intermediate moult taking place in this situation.

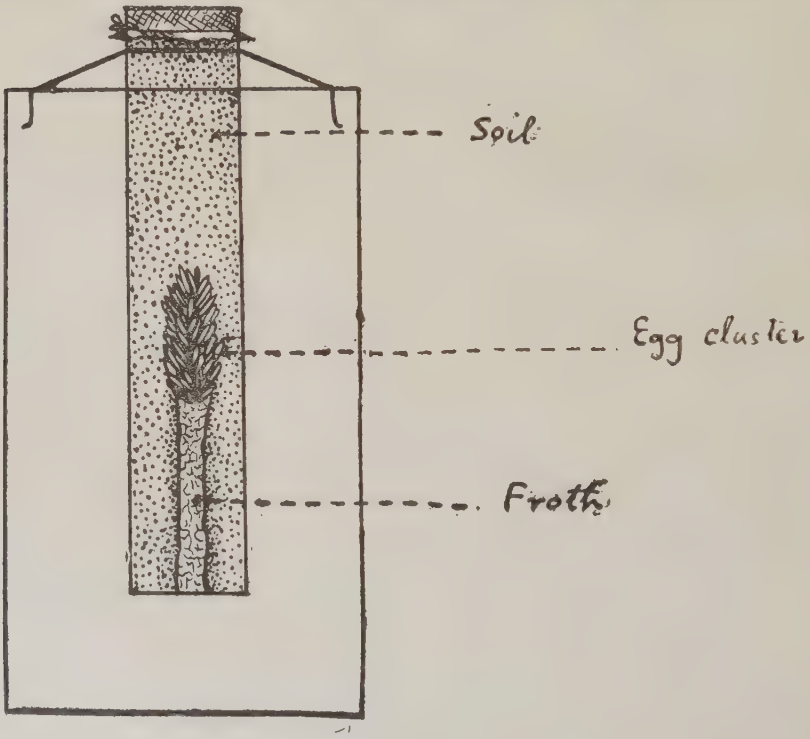

Fig. 2.

The larvae did not work their way upwards as should have happened if negative geotropism had been the determining factor; they all bored their way downwards through the froth.

Experiment 3.—An egg-pod deposited on 7th April 1931 was removed from the soil on 15th April 1931 and placed in a tube made of celluloid, 14 cm. long and 2·4 cm. in diameter (Fig. 3). The egg-pod was 3 cm. long. The tube was placed over a glass chimney (as in experiment 2) in such a manner that the normal upper end of the egg-pod was uppermost and was covered with 4 cm. of sand. At the lower end of the pod a cork (cork No. 1) with a hole in the centre was placed to keep the egg-pod in position. Through this hole in the cork, the original froth column was introduced so as to come in contact with the eggs at the normal lower end of the egg-pod. Round this frothy plug, soil was put and another cork (cork No. 2) with a hole introduced at the lower end of the tube to keep this soil in position.

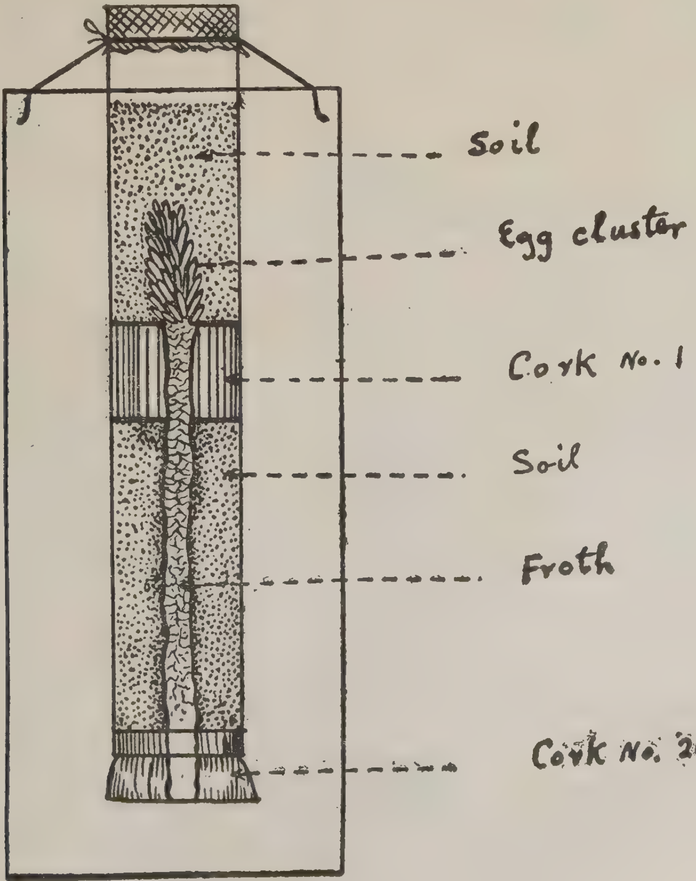

Fig. 3.

The eggs hatched on 19th April 1931 and all the larvae worked their way downwards and passed through the hole in the upper of the two corks. Most of them went round and round in a circle in the space just below this cork. Two of them had almost reached the lower cork, none of them succeeding in finding an opening through the lower cork. None of the larvae went to the upper end of the egg-pod.

This experiment shows that if the froth column is removed from the normal upper end of the egg-pod and placed at the normal lower end of the pod, then the vermiform larvae bore through it and work downwards instead of going upwards.

Experiment 4.—A four-way celluloid tube was prepared. The diameter of the tube was one inch and each limb was 2½ in. long. The four limbs of the cross so formed were marked A, B, C and D (Fig. 4). An egg-pod (without froth) which had been laid in soil in nature (Shergarh in Montgomery District on 15th August 1931) was placed at 12 noon in the central space in the direction of the tube A-B. Two centimetre thick layer of moist soil was put in the four limbs around the egg-pod and the tube placed horizontally. Muslin was tied at the free ends of the tubes.

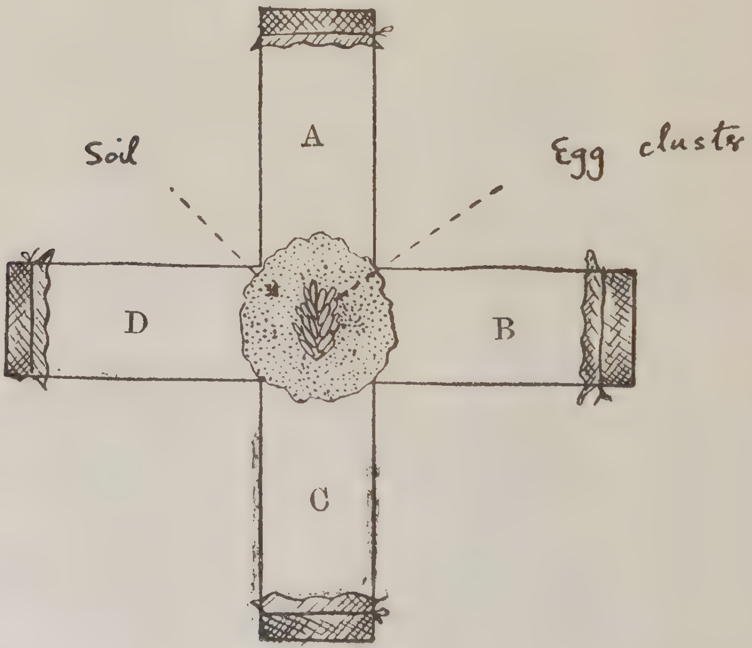

Fig. 4.

Hatching started on the same day at 13 hours and by 18 hours the hoppers had worked their way through the soil into the different limbs of the tube as follows :–

Limb A	6 hoppers.
,, B	4 hoppers.
,, C	7 hoppers.
,, D	3 hoppers.

This experiment shows that if an egg-pod is placed in the soil in such a way that the pressure of soil on all sides is the same, then the larvae after hatching may take any direction.

It has been seen that sometimes eggs are laid in vertical clay-cliffs, locally known as *khundars*, and these burrows being almost at right angles to the surface of the cliff are horizontal in position. In such cases the larvae work their way horizontally and drop down at the foot of the cliff. The larvae hatching out from such a position have actually been observed.

CONCLUSIONS

From these experiments we have arrived at the conclusion that light and gravity play no part in directing the outward movement of the emerging larvae from the soil.

The vermiform larvae after hatching take the path of least resistance and this path, in nature, is provided by the frothy plug deposited at the upper end of the egg-pod. It is through this plug that the larvae work their way up. In other words the direction of the movement of the larvae is determined by pressure ; they move in the direction the pressure is least.

SUMMARY

The existing idea that the movement of the larvae of locusts out of soil is determined by positive phototropism or negative geotropism of the larva is not correct, the direction of their movement is determined by pressure. They move in the direction of least pressure, or in other words they follow the path of least resistance.

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STUDIES IN PLANT BREEDING TECHNIQUE

I. AN ANALYSIS OF THE EFFICIENCY OF SELECTION METHODS USED IN THE IMPROVEMENT OF MALVI COTTON

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(Received for publication on 11th February 1936)

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I. INTRODUCTION

The work here discussed was commenced at the Institute of Plant Industry by Mr. (now Sir) A. Howard and Mrs. Howard in 1925. The aims they had in view are given in their account of the first five years' work of the Institute [Howard and Howard, 1929], and were for Malvi cotton as follows ; " the isolation of a robust, rapidly maturing type with a fibre capable of spinning at least twenty counts which will be suitable for dry cultivation on the Malwa plateau ".

At that time it was believed that the Malvi crop had suffered a serious deterioration as a result of mixing with imported *roseum* seed, and consequently some trouble was taken to obtain seed from isolated tracts where mixing was less easy as a nucleus stock for improvement. Kannod in the Nemawar district of the Holkar State was chosen as such an isolated tract. While geographically it belongs to the Nimar tract, from the point of view of crop ecology it appears to belong to Malwa, and it grows a Malvi type of cotton. Owing to the lack of communication between Nemawar and neighbouring regions of the Central Provinces it was believed that little seed exchange had taken place. It has since been found that the invasion of *roseum* into Malwa has been of negligible importance.

The unselected mixed Malvi crop varies in halo-length from 14 to 22 mm., gins on an average about twenty-nine per cent and has a spinning value of 10-12 H. S. W. C., on the Indian Central Cotton Committee's Technological Laboratory's scale. It is also somewhat late, and the third picking is often lost when frosts occur in mid-January.

II. MATERIAL AND RESULTS

A foundation stock was built up by single-plant selection of Malvi type plants, (1) in 1924-25 direct from cultivators' fields in various villages up to about twenty miles from Indore, and (2) in 1925-26 from Institute fields sown with seed from Kannod.

By progeny-row breeding a series of strains was established, and these were steadily reduced in number by selection both between and within strains until in 1932-33, five remained. Yield testing was commenced in 1931-32, when a replicated, but not randomised trial was carried out with ten strains. In 1932-33, and 1933-34, the five remaining strains were tested against local in randomised block trials in various parts of Malwa, and as a result three more strains were discarded. In 1934-35 the remaining two strains were tested throughout Malwa against local and such improved varieties from neighbouring provinces as appeared likely to do well under black soil conditions, and were proved to be greatly superior both to the local control and to all possible outside competitors. In 1934-35 seed of one of the two final strains, Malvi 1 was distributed to member states in Malwa, and in 1935-36 seed of both strains, Malvi 1 and Malvi 9, was distributed. Further distribution will be of Malvi 9 only. Spinning tests were carried out by the Indian Central Cotton Committee's Technological Laboratory on these two strains with the local as control, in 1932-33, 1933-34, and 1934-35. Data for commercial characters of Malvi 1, Malvi 9 and local (control) are summarised in Table I for the years 1932-33 to 1934-35, during which time all field trials were carried out in randomised replicated layouts.

TABLE I

Commercial characters of Malvi 1, Malvi 9 and local

Strain	No. of trials	Halo length (mm.)	Ginning per cent	Yield (lbs. per acre)	Percentage picked before frost	Spinning tests	
						No. of tests	H.S.W.C.
1932-33—							
Local	4	19·8	28·7	484	91·1	3	12·2
Malvi 1	4	21·7	29·3	580	93·7	3	13·3
Malvi 9	4	22·5	32·3	554	91·7	3	15·5
1933-34—							
Local	6	21·3	29·6	330	85·6	2	11·5
Malvi 1	6	22·9	31·0	409	91·6	5	12·2
Malvi 9	6	23·2	33·3	413	89·6	4	16·8
1934-35—							
Local	7	23·6	28·5	325	63·3	..	..
Malvi 1	7	23·9	29·3	332	74·3	2	14·0
Malvi 9	7	24·3	31·9	357	65·1	2	20·0
Mean increase over local—							
Malvi 1	..	1·3	0·9	60·7	6·5	..	0·9
Malvi 9	..	1·8	3·6	61·7	2·1	..	4·3

Inspection of Table I shows that both strains are better than the local control in all characters, and, in all characters except earliness and possibly yield, Malvi 9 is better than Malvi 1. The data are summarised from records of seventeen trials, in most of which the differences observed were significant, so that the differences in the means can be relied on as representing real differences between the strains. The increase in value to the cultivator, obtainable by substituting Malvi 9 for local may be estimated at about thrity-five per cent with no allowance for premium due to enhanced spinning value and about fifty per cent including premium for enhanced spinning value. The fact that marked improvement has been obtained in all the major commercial characters has led to a widespread and insistent demand for seed, and the difficulties usually experienced in distributing seed of a strain dependent for its superiority on receiving a premium for high spinning value are not being encountered. In addition, prominent representatives of the Indore and Bombay mill industries have urged its rapid spread as promising an improvement of first importance in the quality of the Malvi crop. As a plant-breeding project, therefore, the work under discussion is abundantly justified by its results.

The interest of the selection records lies in the information they provide concerning the efficiency of the methods adopted. Two strains have been isolated which are markedly superior to the local. Does this achievement represent the maximum possible exploitation of the material examined, or were there genetic potentialities in it which the methods adopted were not sufficiently selective to reveal ?

III. METHODS

Of the characters studied, vigour was given first importance throughout the period and the number of bolls per plant was ranked second. Of other yield characters weight of *kapas* per plant, and weight of lint per plant and lint index were studied and selections based on them in some seasons. Earliness was estimated throughout by the date of opening of the first boll, and in some years by the date of the first flower or the date of the last picking. It was considered of less importance than yield. In general, only those families which were judged in the field as satisfactory in vigour, yield and earliness were carried on for study of lint characters in the laboratory.

Of the lint characters studied, "feel" (fine or coarse) and ginning percentage were considered together, as they were found to be to a large extent mutually exclusive, and it was desired to combine as far as possible a soft lint with a high ginning percentage. These two characters were ranked as of greater importance than lint length which was judged by maximum halo-length.

In Tables II to V are summarised the selection data on four representative characters ; bolls per plant, date of bursting of first boll, ginning percentage, and halo-length, for the period 1927-28 (halo-length from 1926-27) to 1933-34.

In following the course of the strain means for yield and earliness from year to year, it is clear that other factors besides selection have influenced them largely. On bolls per plant the most important environmental influence is the incidence of late rains causing shedding, and it is clear from Table II that the major fluctuations in boll number were due to this factor and not to selection. Late rains affect the date of first boll bursting similarly, and for the same reason. Ginning percentage figures run consistently. Halo-length means gave a large jump between 1927-28 and 1928-29, and differed very little from each other in subsequent years.

The effect of selection will be two-fold : first to isolate from among the foundation strains those which were superior from the beginning, and secondly, to improve the performance of the original strains by favouring their best components. (Lines 6 and 7 in Tables II to V). The intensity of selection can be correspondingly divided into two parts, first the difference between the mean of strains selected for propagation in the next year and the mean of all strains and secondly the difference between the mean of plants selected as parents and the mean of the selected strains to which they belong. (Lines 8 and 9 in Tables II to V).

TABLE II

Number of bolls per plant in Malvi selections

	1927-28	1928-29	1929-30	1930-31	1931-32	1932-33	1933-34
1. Number of strains	38	25	15	11	8	5	5
Means.							
2. All strains examined	10·4	15·4	19·3	7·4	10·3	12·9	8·3
3. Selected strains	11·6	17·5	19·4	7·3	12·0	12·9	..
4. Selected plants	17·0	21·6	28·6	11·0	22·7	16·9	..
5. Five final selected strains	13·8	17·6	20·6	7·4	12·0	12·9	8·3
Increments over previous year.							
6. All strains examined	..	+5·0	+3·9	—11·9	+2·9	+2·6	—4·6
7. Selected strains over their own parents	..	+3·8	+1·8	—12·0	+3·0	+0·9	—4·6
Selection differences.							
8. Selected strains from all strains	+1·2	+2·1	+0·1	—0·1	+1·7	0	..
9. Selected plants from selected strains	+5·4	+4·1	+9·2	+3·7	+10·7	+4·0	..
10. Weather characteristics	Late rains.	No late rains. Slight frost.	No late rains. Frost.	Serious late rains.	Serious late rains.	Late rains.	Late rains. Frost.

The data given for number of bolls per plant (Table II) are so strongly influenced by the incidence of late rains that no appreciable connection can be discovered between the selection intensities (lines 8 and 9) and the increments in the means in the following year (lines 6 and 7). That selection has been effective, however, is shown by the fact that in four out of the five comparable years, the mean number of bolls per plant was greater for the five strains finally selected than for all strains examined in the same years. It is clear that a group of strains with a higher mean-boll-number has been isolated, though it is not possible to say whether these strains have improved under the influence of selection.

TABLE III

Date of bursting of first boll, in Malvi selections (in days from October 1st)

	1927-28	1928-29	1929-30	1930-31	1931-32	1932-33	1933-34
1. Number of strains	..	24	15	11	8	5	5
Means.							
2. All strains examined . .	..	43·6	20·9	40·0	55·0	33·8	29·3
3. Selected strains .	22·4	44·0	20·5	39·7	55·4	33·8	..
4. Selected plants .		43·4	21·4	38·6	52·5	32·5	..
5. Five final selected strains . .	22·9	44·7	21·6	38·7	55·4	33·8	29·3
Increments over previous year.							
6. All strains examined . .	..	..	—22·7	+19·1	+15·0	—21·2	—4·5
7. Selected strains over their own parents . .	..	..	—23·1	+19·5	+15·3	—21·7	—4·5
Selection differences.							
8. Selected strains from all strains	..	+0·4	—0·4	—0·3	+0·4	0	..
9. Selected plants from selected strains . .	..	—0·6	+0·9	—1·1	—2·9	—1·2	..
10. Weather characteristics .	Late rains.	Very heavy early and August rains. No late rains.	No late rains.	Serious late rains.	Serious late rains.	Late rains.	Late rains.

NOTE.—Negative signs indicate earliness and positive signs lateness.

There is no evidence of any improvement in the data given in Table III for the date of bursting of the first boll, and in view of the small selection differences— not even always in the right direction—no improvement could reasonably be expected.

TABLE IV

Ginning percentage in Malvi selections

	1927-28	1928-29	1929-30	1930-31	1931-32	1932-33	1933-34
1. Number of strains	38	25	11	10	5	5	2
Means.							
2. All strains examined . .	24·6	28·6	29·0	28·5	31·0	31·3	32·3
3. Selected strains .	25·0	28·9	29·7	30·8	31·0	31·0	..
4. Selected plants	26·4	31·3	29·0	33·2	33·4	32·7	..
5. Five final selected strains . .	28·4	31·5	32·3	30·8	31·0	31·3	..
Increments over previous year.							
6. All strains examined . .	..	+4·0	+0·4	—0·5	+2·5	+0·3	+1·3
7. Selected strains over their own parents . .	..	+3·6	+0·1	—1·2	+0·2	+0·3	+1·3
Selection differences.							
8. Selected strains from all strains	+0·4	+0·3	+0·7	+2·3	0	—0·3	..
9. Selected plants from selected strains . .	+1·4	+2·4	—0·7	+2·4	+2·4	+1·7	..

The ginning percentage data (Table IV) show a steady year-by-year improvement which can safely be ascribed to selection. One anomaly is evident. The increment of 1928-29 (all strains) over 1927-28 (all strains) was 4·0, whereas the total selection difference in 1927-28 is only 1·8. The increment is therefore considerably greater than can reasonably be ascribed to selection. Apart from this exception the yearly increments are roughly proportional to the total selection difference, and in 1930-31 there was a drop in mean ginning percentage (all strains) following a zero net selection difference in 1929-30. The five finally selected strains were in all four years superior to the mean of all strains, and within themselves they show no appreciable year-by-year improvement, indicating that most at least of the selective gain has been due to the isolation of strains superior to the average from the beginning, and not to improvement within strains.

TABLE V

Lint-length in Malvi selections (halo-length in mm.)

	1926-27	1927-28	1928-29	1929-30	1930-31	1931-32	1932-33	1933-34
1. Number of strains	34	29+6	25	11	11	5	5	5
Means.								
2. All strains examined . .	20·0	20·1	23·2	22·7	22·9	22·7	22·3	23·1
3. Selected strains	20·1	20·6	23·3	22·7	23·1	22·7	22·3	..
4 Selected plants	20·8	21·5	23·8	23·0	23·8	23·2	23·3	..
5. Five final selected strains . .	20·8	20·2	23·2	23·1	23·1	22·7	22·3	23·1
Increments over previous year.								
6. All strains examined . .	..	+0·1	+3·1	—0·5	+0·2	—0·2	—0·4	+0·8
7. Selected strains over their own parents . .	..	0	+2·6	—0·6	+0·2	—0·4	—0·4	+0·8
Selection differences.								
8. Selected strains from all strains	+0·1	+0·5	+0·1	0	+0·2	0	0	..
9. Selected plants from selected strains . .	+0·7	+0·9	+0·5	+0·3	+0·7	+0·5	+1·0	..

There were small positive selection differences in halo-length in all years (Table V). Apart from the large jump between 1927-28 and 1928-29 mentioned above, changes in mean halo-length from year to year were small, and there is no evidence of any upward trend which could be ascribed to the influence of selection. It is not possible to ascribe the difference between 1927-28 and 1928-29 means to the effect of selection, as it is much larger than the corresponding selection difference.

IV. DISCUSSION

On the whole changes which can reasonably be ascribed to the influence of selection are disappointingly small, although two very superior strains have in fact been isolated. Environmental influences appear to have masked a real selective effect on boll number. Improvement in earliness would not be expected with the negligible selection differences employed, even if environmental fluctuations had been less extreme. The halo-length results are the most disappointing. A steady, though small, selection difference was maintained throughout, and environmental influences were not serious, but no improvement ascribable to selection was obtained.

For the improvement of plant breeding technique it is most important to discover whether the potentialities of the original material were fully exploited. If the original genetic variance was so low that the actual improvement obtained was the maximum possible, no improvements in technique would affect the achievements of selection. If, however, considerable genetic variance existed in the original population and remained undetected by the selection methods adopted, the improvement of breeding technique may be expected to make a corresponding improvement in the achievements of selection.

In the 1934-35 season progeny-rows of the two finally selected strains, Malvi 1 and Malvi 9, were laid out in two experiments in randomised plots replicated ten times, each plot consisting of a single row of five plants. From the data obtained from these experiments it was possible to estimate the genetic variance for various characters existing between progenies of two strains self-fertilised for eight generations. Data are given in Tables VI and VII.

TABLE VI

Means, standard errors, and significance of differences in replicated progeny-rows of Malvi 1, 1934-35

Progeny numbers.	1	2	3	4	5	6	7	8	9	10	Per cent st. error	Significant diff.	Order of merit
Total yield of *kapas* in gms. per progeny.	558·9	629·1	531·1	627·2	535·0	474·0	641·7	498·0	629·3	660·8	14·9	...	10, 7, 9, 2, 4, 1, 5, 3, 8, 6
Total stand per progeny	41	45	43	47	33	38	39	36	45	45	7·1	8·34	4, 2, 9, 10, 3, 1, 7, 6, 8, 5
Total fruiting nodes per progeny.	2202	1931	1538	2021	1669	1882	2048	1837	2052	2089	10·5	...	1, 10, 9, 7, 4, 2, 6, 8, 5,3
Mean ginning per cent per progeny.	27·8	30·1	28·5	31·9	27·3	27·3	27·1	27·1	27·7	27·8	1·8	1·47	4, 2, 3, 10, 1, 9, 5, 6, 7, 8
Mean vigour per progeny	—1·2	—1·5	—1·0	—0·9	—1·0	—1·1	—0·7	—1·0	—1·1	—1·2	18·7	...	2, 1, 10, 6, 9, 3, 5, 8, 4, 7
Mean lint-length in mms. per progeny.	22·9	22·9	22·4	22·6	22·7	23·2	23·3	23·1	22·9	22·6	0·99	...	7, 6, 8, 1, 2, 9, 5, 4, 10, 3
Mean node number per progeny.	11·54	10·13	11·29	10·81	10·50	11·60	11·87	11·78	12·12	12·05	2·1	0·67	2. 5, 4, 3, 1, 6, 8, 7, 10, 9

TABLE VII

Means, standard errors, and significance of differences in replicated families of Malvi 9, 1934-35

Family numbers.	13	15	17	19	20	St. error per cent for families	Sig. diff.	St. errors per cent for progenies	Families with progenies differing significantly	Remarks
Mean yield of *kapas* in gms. per family per plot	41·14	40·58	48·72	52·93	50·03	3·8	4·7	11·5	...	19, 20, 17 13, 15
Final mean stand per family per plot	4·17	4·38	4·33	4·55	4·70	3·5	0·38	5·3	13, 15, 17, 19, 20	20, 19, 15, 17, 13
Mean ginning per cent per family	31·97	32·08	32·48	32·63	32·26	0·5	0·468	1·9	...	19, 17, 20, 15, 13
Mean fruiting nodes per family per plot	152·28	157·24	178·57	186·43	180·69	2·5	12·0	7·7	...	19, 20, 17, 15, 13
Total vigour per family	+9	—4	—56	—51	—50	35·8	30·8	90·0	15, 17, 19, 20	17, 19, 20, 15, 13
Mean lint-length per family	21·04	21·28	21·28	21·17	21·89	0·55	0·33	1·5	...	20 17, 15, 19, 13
Mean node number per progeny	11·4	11·5	11·8	10·5	11·3	1·4	0·5	32·8	17, 19	19, 20, 13, 15, 17
Percentage yield before frost per family per plot	63·9	65·0	59·0	60·0	58·5	2·9	5·0	8·8	...	15, 13, 19, 17, 20

The ten progenies of Malvi 1 were from sister plants of the 1933-34 crop. Between them there were demonstrated significant differences in total stand, ginning percentage, and node number.

In Malvi 9, ten progenies were grown from each of five different families belonging to two different sub-strains. The fifty progenies were randomised. and replicated ten times, each progeny contributing ten single-row plots of five plants each. Significant differences between families were demonstrated for every character studied, even including vigour for which the standard error was as high as thirty-six per cent. Significant differences between progenies within families were demonstrated for final stand, vigour and node number. Yield of *kapas*, ginning percentage, fruiting nodes, vigour, lint-length and percentage of yield before frost are all characters directly affected by the selection practised, while final stand and node number are not. In the single family of Malvi 1 studied genetic variance remains for both the characters not influenced by the selection practised, and also for ginning percentage, in which selection has been most effective. In Malvi 9 genetic variance exists between families for all characters studied, and within families for the two not affected by selection, and also for vigour.

The genetic variance between families is an index of the genetic variance existing in the single plant of the 1928 progeny of Malvi 9 from which all families were descended, while the genetic variance existing between progenies of the same family in 1934-35 is an index of the genetic variance within the five plants from which they sprang in 1932. A comparison of the extent of genetic variance between and within families shows that a great deal was lost in the four years, 1928-32. This is presumably due to increasing homozygosis. It is abundantly clear that there was a large amount of genetic variance available in 1928 for characters on which selection was being practised. It was not exploited, and remained undetected until more sensitive methods were adopted in 1934-35. Much of it was lost from within families by increasing homozygosis in the intervening period, but sufficient remains to provide material for further selection in some characters, notably ginning percentage in Malvi 1, the character most successfully influenced by selection hitherto, and vigour in Malvi 9.

The method of randomisation and replication of progeny rows provides a measure, not only of the mean differences between progenies, but also of the errors to which these differences are subject, and enables the plant breeder not only to choose the best strains for further propagation, but also to decide with confidence whether their superiority is due to genetic or environmental causes. Its sensitiveness as a selection method is abundantly clear from the data presented in Tables VI and VII. If such potentialities for further improvement can be demonstrated in material on which selection has already been practised with great success, it is reasonable to expect that with unselected material the speed and extent of improvement will be greater.

There remains for consideration the question, to what is the improvement of the selected strains over the local due, if not to the selection method ? There appears little doubt that it must be ascribed to that indefinable judgment of unmeasured characters, the necessity for which makes plant breeding as much an art as a science. It is to this plant-breeding sense that the great breeders of the past have owed their success. While it is hoped that this discussion makes it clear that greatly superior statistical weapons can now be placed in the breeder's hands, it should be emphasised that no improvement in statistical methods, however great, can absolve the breeder from the trouble of learning his material. He must know his plants as he knows his own brother if he is to exercise that judgment which is—and will remain—the secret of his success.

V. Summary

(1) The aims, methods, and achievements of selection in Malvi cotton are summarised.

(2) Two strains superior to the local mixture in all characters have been isolated and are being distributed.

(3) The methods and achievements of selection are analysed, and it is concluded that on the whole the changes ascribable to selection have been disappointingly small in spite of the superiority of the finally selected strains.

(4) Data from randomised, replicated progeny-row experiments on the selected strains are summarised and show clearly that the methods of selection adopted were not sufficiently critical to detect a large amount of genetic variance which was present in the material.

(5) The advantages for selection work of replication and randomisation of progeny rows are pointed out.

(6) The success achieved is ascribed primarily to the breeder's judgment and not to the selection methods. The paramount importance of the breeder's art is emphasised, and it is pointed out that no improvement in selection technique will free him from the need to gain that familiarity with his material which makes the successful exercise of his judgment possible.

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IDEAL SIZE AND SHAPE OF SUGARCANE EXPERIMENTAL PLOTS BASED UPON TONNAGE EXPERIMEMTS WITH Co. 205 AND Co. 213 CONDUCTED IN PUSA

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(Received for publication on 21st April 1936)

(With Plate XLI and two text-figures)

INTRODUCTION

The importance which the question of plot size and shape bears in relation to field experiments with different crops has now been realised by all agricultural workers. It has now become more than necessary to standardize for several experimental crops grown under different conditions such as irrigated or rain-fed, a suitable plot size and shape for the experimental plot which will reduce the standard error of the experiment to a practical minimum. Field experiments in other countries planned on modern lines of field technique have shown that the plot size and shape do exercise a considerable influence on the standard error of the experiment and that a particular size or shape of an experimental plot is always reached for a particular crop on a particular soil when the distribution of plot yields (subjected to the same treatment, other conditions of experiment remaining identical) can be ascribed to chance or chance alone. The question of economy in land and labour demands that the size of plot in the experiment should be as small as possible consistent with efficiency, and with a crop like sugarcane which is expensive to grow and occupies the land for a year, the need for fixing up a suitable plot size for experiments is acutely felt.

With such aims before us, the cutting experiments with Co. 205 and Co. 213, detailed subsequently, were undertaken to find out the most suitable plot size and shape for sugarcane experiments under unirrigated conditions. Up till now no work on this line has been done in India with sugarcane which is now one of the most important economic crops in India. The importance of an investigation of this nature to agricultural experimenters can, therefore, hardly be over-emphasised.

It is equally important to recognise the need for a correct statistical technique for problems of this kind, particularly as most of the agricultural farms in India are now making efforts to standardise the size and shape of their experimental plots.

I. Tonnage experiments with Co. 205 in 1932 at Pusa

Materials and methods.—A tonnage experiment on sugarcane, Co. 205, unirrigated, was conducted in Harpur Jhilli in 1932. Forty-two rows of cane with a space of three feet between rows were selected and cut by sections, each section being 30 ft. 3 in. long. Thus the yield figures of plot sizes 30 ft. 3 in. × 3 ft. (*i.e.*, 1/480 acre each), numbering 840 such plots in all, were available for statistical analysis ; for convenience the data of yields of the first forty rows were also considered separately.

Experimental results.—The yields of several plot sizes were combined in all possible ways, and the coefficient of variation of each size was worked out (*i.e.*, the percentage ratio of standard deviation to mean) (the details of the method have been explained in subsequent pages).

Table I

Analysis of the data of tonnage experiments with Co. 205 sugarcane in 1932

Plot size	Area (acre)	Mean yield per plot (in lbs.)	S. D.	Coef. of variation $\left(=\frac{\text{S.D.}\times 100}{\text{mean}}\right)$	*Relative precision corrected for number of replications
I. (*a*) 30 ft. 3 in. × 3 ft., 42 rows.	1/480	90·7	31·55	34·8	100
30 ft. 3 in. × 3 ft., 40 rows.	1/480	92·65	30·55	32·9	107
II. (*a*) 30 ft. 3 in. × 6 ft., 40 rows.	1/240	185·3	46·9	25·3	91
30 ft. 3 in. × 6 ft., 42 rows.	1/240	181·4	47·4	26·1	89
(*b*) 60 ft. 6 in. × 3 ft., 42 rows.	1/240	181·4	53·9	29·7	69
60 ft. 6 in. × 3 ft., 40 rows.	1/240	185·3	51·8	28·0	74
III. (*a*) 60 ft. 6 in. × 6 ft., 40 rows.	1/120	370·6	83·2	22·5	58
60 ft. 6 in. × 6 ft., 42 rows.	1/120	362·8	89·6	24·7	49
(*b*) 121 ft. × 3 ft., 42 rows	1/120	362·8	92·4	25·5	47
121 ft. × 3 ft., 40 rows	1/120	370·6	85·26	23·0	55
(*c*) 30 ft. 3 in. × 12 ft., 40 rows.	1/120	370·6	72·2	19·5	76

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*For the method of calculation of this column, see pages 700-701.

TABLE I—*contd.*

Plot size	Area acre)	Mean yield per plot (in lbs.)	S. D.	Coef. of variation $\left(=\frac{\text{S.D.}\times 100}{\text{mean}}\right)$	*Relative precision corrected for number of replications
IV. (*a*) 60 ft. 6 in. × 9 ft., 42 rows.	1/80	544·2	124·81	22·9	37
V. (*a*) 121 ft. × 6 ft., 40 rows	1/60	741·2	145·2	19·6	38
121 ft. × 6 ft., 42 rows	1/60	725·6	167·2	23·0	29
(*b*) 60 ft. 6 in. × 12 ft., 40 rows.	1/60	741·2	140·2	19·0	40
(*c*) 30 ft. 3 in. × 24 ft., 40 rows.	1/60	741·2	130·8	17·6	47
VI. (*a*) 60 ft. 6 in. × 18 ft., 42 rows.	1/40	1088·4	210·54	19·3	24
(*b*) 121 ft. × 9 ft., 42 rows	1/40	1088·4	220·2	20·2	25
VII. (*a*) 60 ft. 6 in. × 24 ft., 40 rows.	1/30	1482·4	279·5	18·85	22
(*b*) 121 ft. × 12 ft., 40 rows	1/30	1482·4	236·96	16·0	28
VIII. (*a*) 121 ft. × 18 ft., 42 rows	1/20	2176·4	382·32	17·6	16
IX. (*a*) 151 ft 3 in. × 18 ft., 40 rows.	1/15	2779·5	502·4	18·1	13
(*b*) 302 ft. 6 in. × 9 ft., 40 rows.	1/15	2779·5	501·06	18·0	13

The plot size giving the minimum coefficient of variation was considered to be a suitable size for an experimental plot.

The plot size with a minimum coefficient of variation in this case was found to be between 1/30 and 1/20 acre with dimensions 121 ft.×12 ft. and 121ft. × 18ft. respectively. The results also showed generally that as the shape of the plot got narrower, the coefficient of variation gradually diminished.

The chief defect of the experiment was found, however, to be that the soil variation between plot and plot or row and row was highly marked, as Table I of the coefficient of variation for different plot sizes, and of rapidly decreasing efficiency corrected for the number of replications, clearly shows. The coefficient of variation, however, went down from thirty-five per cent to sixteen per cent as the plot size increased, but in no case was it less than sixteen per cent which itself was indeed a very high figure with a corresponding precision of twenty-eight per cent. It was therefore decided that the experiment should be repeated next year, but with a more popular variety of cane Co. 213, on soils of fairly uniform fertility.

II. Tonnage experiments with Co. 213 in 1933 at Meghaul (Monghyr)

Co. 213 is recognised as the standard cane of the white sugar tract and represents probably the most generally grown cane crushed in sugar factories on the tract. All important cane experiments conducted at Pusa are laid down under this variety or Co. 210 and it was therefore felt advisable to conduct this experiment with Co. 213.

General description and history of Co. 213

Detailed description and history of Co. 213 have been given by Venkatraman and Vittal Rao [1928].

This cane, which has attained such universal success in North India was bred by Dr. C. A. Barber at Coimbatore and was first sent by him to Pusa in 1918-19 together with a lot of other seedlings. It was tested here in small plots for two years and then selected together with Co. 210 and Co. 214 for the first mill trial by the Secretary, Sugar Bureau, Pusa in December, 1922 [Sayer, 1923]. The test was conducted at Ryam, one of the sugar factories of Messrs. Begg Sutherland & Co. near Darbhanga by kind courtesy of the firm.

TABLE II

Chemical analysis of the first mill juice

Date of crushing	Variety	Brix	Sucrose	Purity
27th December 1922 . .	Co. 213	17·63	14·52	82·86

The fibre contents in Co. 213 and Co. 210 were found to be 16·84 and 15·66 per cent respectively lower than Co. 214. The poor sucrose percentage of Co. 213 in December is accounted for by the fact that it is a late-ripening cane, reaching its maximum at the end of January. The following analysis of juice was given by the Imperial Agricultural Chemist, Pusa, in 1933 :—

TABLE III

Chemical analysis of Co. 213 in January 1933

Variety	Date of analysis	Average weight of cane in lb.	In juice		
			Juice	Sucrose	Purity
Co. 213	21st Jan. 1933	1·68	Per cent 63·43	Per cent 17·39	Per cent 86·83

Having proved its high value in the mill trial and having displayed a remarkable tonnage capacity and excellent agricultural habit on an estate scale, its cultivation was extended over a larger area in 1922-23 by the Secretary, Sugar Bureau, in order to supply seed to cane growers of the Province. The cane is now cultivated in all provinces of North India; it occupies the first place as regards area under cultivation, in Bihar, Bengal and the United Provinces. It is also grown on a fairly large area in the Punjab, Madras, Bombay and Assam. The average tonnage of this cane on the Pusa Farm has been about 600 maunds (22·0 tons) per acre.

MATERIALS AND METHODS

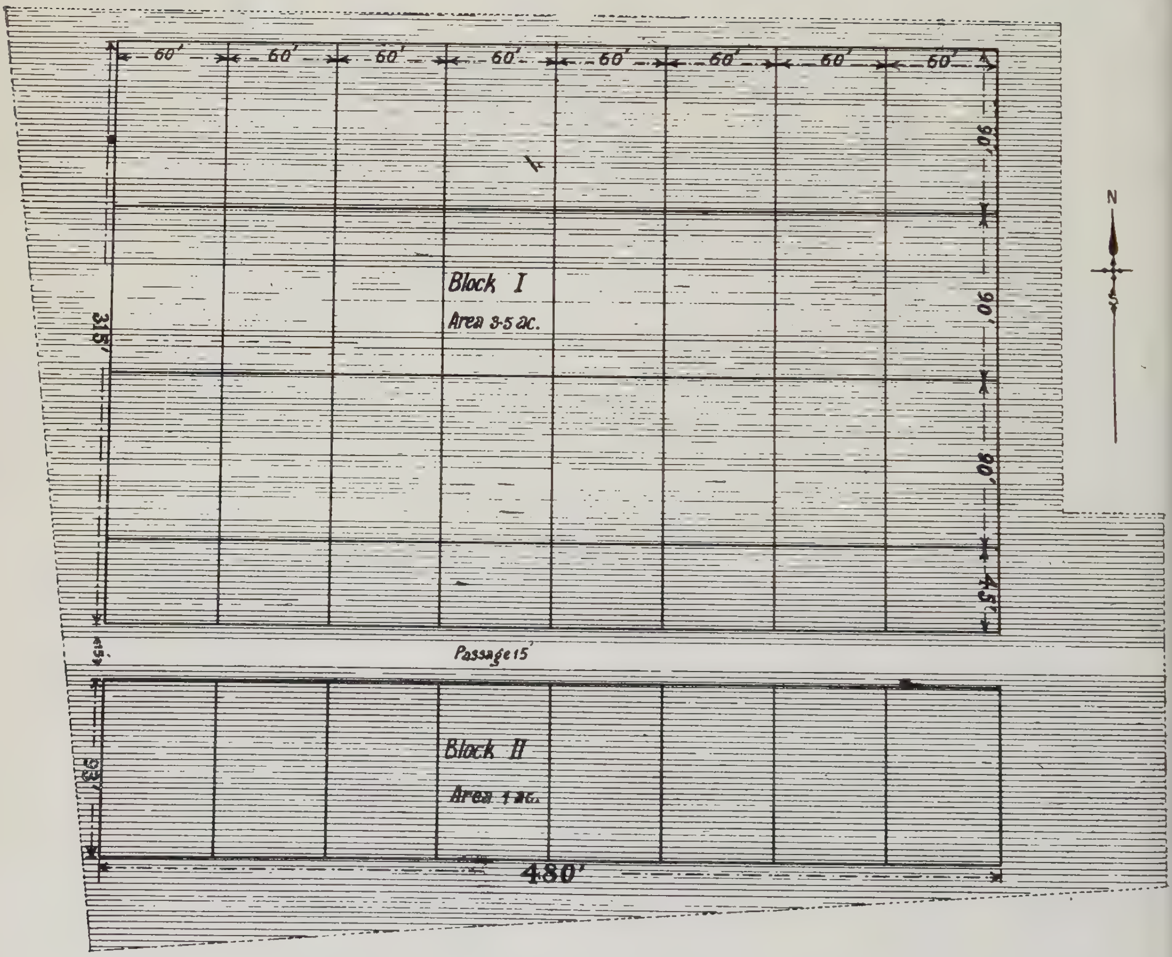
The experiment was conducted at Meghaul, District Monghyr, on a six acre plot of Co. 213 cane very kindly made available for the purpose by Mr. C. Atkins, Manager, Dowlatpore Agricultural Concern, without whose valuable assistance it would have been impossible to conduct the experiment.

The land was under a regular sugarcane rotation in previous years; cultural methods, manuring and other operations were uniform all over the area. The soil throughout the field was a good class of cane loam—a typical *sumbi*. The field was planted on the 12th February, 1932; the setts were planted eye to eye in rows three feet apart with six feet of the ends doubled. Manuring was done at the rate of 400 maunds (14.7 tons) farmyard manure per acre which was purchased at the rate of one anna per maund locally. The operation of earthing up the rows was duplicated, once before and once after the break of monsoon. The cane was not irrigated, and the record of rainfall for the year of its growth is given below :—

	Inches
1st January to 31st May	*Nil*
June	5·49
July	4·49
August	9·18
September	5·34
October	0·99
November	3·66
Total .	29·15
Average annual rainfall	47

The crop presented a very uniform growth and there was very little lodging.

The cutting of the canes was started on the 12th February, 1933. Before the commencement of the experiment, a passage of fifteen feet wide had been cut through the field to facilitate inspection. The crop was thus located in two blocks (Blocks I and II) separated by a temporary road. These blocks were


60'
60'
60'
60'
60'
60'
60'
60'
90'
90'
90'
45'
315'
Block I
Area 3·5 ac.
Passage 15'
15'
Block II
Area 1 ac.
93'
480'
N
S

Plan of Co. 213 sugarcane field at Meghaul, Monghyr, showing lay-out of the cutting experiment.

trimmed on all sides by removing the border crop ranging from nine to thirty feet in width and shaped into two perfect rectangular blocks. The blocks then measured 480 ft. × 315 ft. (three acres and a half) and 480 ft. × 93 ft. (one acre) as shown in the plan (Plate XLI).

After the removal of the border crop, each row measured 480 feet in length and there were 136 such rows, each three feet apart, running east to west in an entire area of four acres and a half. The next step was to divide each row into eight equal sections; the ultimate plot obtained in this way consisted of one row a-piece, and this time it was 60 ft. × 3 ft. = 1/242 acre. These plots were cut, stripped and weighed separately guarding against all possible sources of error. The total number of weighings carried out in this way was 1,088, and it took sixteen days to finish the entire work. The weight of canes from each plot was originally recorded in pounds on two platform weighing machines which proved very useful for the purpose. Appendix gives the yields in pounds of the cuttings in several rows.

The crop cut out at an average weight of 1,000 maunds (36·7 tons) per acre, thus proving that such tonnages can be obtained in a year of short rainfall with proper manuring and soil management without any irrigation. It may also be noted that no artificials of any kind were applied to the crop.

STATISTICAL METHODS ADOPTED AND RESULTS

Instead of dealing with the entire field, four sub-blocks of thirty rows each, as shown below, were taken out for the purpose of this statistical study :—

	Rows
Sub-block I (Block I)	1—30
„ II („)	31—60
„ III („)	61—90
(The other rows 15 in number were omitted.)	
Sub-block IV (Block II)	106—135
(The last row was omitted.)	

The omission of the rows was with the object of securing compact blocks of thirty rows each.

The soil variation in this experiment was found much less marked than in the case of the experiment last year, as the coefficient of variation had in no case exceeded 10·4 per cent. Though we could possibly have all combinations of plot sizes varying from 1/242 acre to 120/242 acre, the plot sizes beyond 16/242 acre were omitted as the frequency was small. We are thus left for each sub-block with thirteen different plot sizes as shown in Tables IV-VII; the plot sizes with frequencies of multiples of five alone being considered for reasons explained in page 696.

TABLE IV

Analysis of data of tonnage experiments with Co. 213 sugarcane in 1933

Sub-block No.	Cuts × rows	Plot dimensions	Ratio of length to width	Plot acreage	Total No. of plots	Mean yield in lb. per plot	S. D. (after elimination of soil variance)	V = Coef. Var. $\left(\frac{\text{S. D.} \times 100}{\text{Mean}}\right)$	S. E. of V.
I	1×1	60 ft.× 3 ft.	20	1/242	240	340·8	31·4	9·2	0·4234
	1×2	60 ft.× 6 ft.	10	2/242	120	681·6	41·2	6·0	0·3925
	2×1	120 ft.× 9 ft.	40	2/242	120	681·6	45·5	6·7	0·4354
	1×3	60 ft.× 9 ft.	6·7	3/242	80	1,022·4	61·4	6·0	0·4760
	4×1	240 ft.× 3 ft.	80	4/242	60	1,363·2	71·0	5·2	0·4760
	2×2	120 ft.× 6 ft.	20	4/242	60	1,363·2	81·64	6·0	0·5497
	1×6	60 ft.×18 ft.	3·3	6/242	40	2,044·8	100·9	4·9	0·5491
	2×3	120 ft.× 9 ft.	13·3	6/242	40	2,044·8	98·6	4·8	0·5378
	8×1	480 ft.× 3 ft.	160	8/242	30	2,726·4	104·7	3·8	0·4914
	4×2	240 ft.× 6 ft.	40	8/242	30	2,726·4	129·5	4·7	0·6082
	4×3	240 ft.× 9 ft.	26·7	12/242	20	4,089·6	178·3	4·4	0·6971
	2×6	120 ft.×18 ft.	6·7	12/242	20	4,089·6	179·6	4·4	0·6971
	8×2	480 ft.× 6 ft.	80	16/242	15	5,452·8	212·8	3·9	0·7131

TABLE V

Analysis of data of tonnage experiments with Co. 213 sugarcane in 1933

Sub-block No.	Cuts × rows	Plot dimensions	Ratio of length to width	Plot acreage	Total No. of plots	Mean yield in lb. per plot	S. D. (after elimination of soil variance)	V = Coef. Var. $\left(\frac{\text{S. D.} \times 100}{\text{Mean}}\right)$	S. E. of V.
II	1×1	60 ft.× 3 ft.	20	1/242	240	338·6	28·6	8·4	0·3861
	1×2	60 ft.× 6 ft.	10	2/242	120	677·2	40·2	5·9	0·3822
	2×1	120 ft.× 3 ft.	40	2/242	120	677·2	41·5	6·1	0·3952
	1×3	60 ft.× 9 ft.	6·7	3/242	80	1,015·8	51·1	5·0	0·3962
	4×1	240 ft.× 3 ft.	80	4/242	60	1,354·4	68·4	5·0	0·4575
	2×2	120 ft.× 6 ft.	20	4/242	60	1,354·4	63·91	4·7	0·4300
	1×6	60 ft.×18 ft.	3·3	6/242	40	2,031·6	85·1	4·2	0·4704
	2×3	120 ft.× 9 ft.	13·3	6/242	40	2,031·6	90·4	4·5	0·5041
	8×1	480 ft× 3 ft.	160	8/242	30	2,708·8	110·7	4·1	0·6602
	4×2	240 ft.× 6 ft.	40	8/242	30	2,708·8	106·5	3·9	0·5043
	4×3	240 ft.× 9 ft.	26·7	12/242	20	4,063·2	116·7	2·9	0·4589
	2×6	120 ft.×18 ft.	6·7	12/242	20	4,063·2	141·0	3·5	0·5541
	8×2	480 ft.× 6 ft.	80	16/242	15	5,417·6	136·4	2·5	0·4568

TABLE VI

Analysis of data of tonnage experiments with Co. 213 sugarcane in 1933

Sub-block No.	Cuts × rows	Plot dimensions	Ratio of length to width	Plot acreage	Total No. of plots	Mean yield in lb. per plot	S. D. (after elimination of soil variance)	V = Coef. Var. $\left(\frac{\text{S. D.} \times 100}{\text{Mean}}\right)$	S. E. of V.
III	1×1	60 ft. × 3 ft.	20	1/242	240	330·2	31·45	9·5	0·4375
	1×2	60 ft. × 6 ft.	10	2/242	120	660·4	47·74	7·2	0·4671
	2×1	120 ft. × 3 ft.	40	2/242	120	660·4	48·74	7·4	0·4803
	1×3	60 ft. × 9 ft.	6·7	3/242	80	990·6	63·25	6·4	0·5081
	4×1	240 ft. × 3 ft.	80	4/242	60	1,320·8	81·39	6·2	0·5682
	2×2	120 ft. × 6 ft.	20	4/242	60	1,320·8	80·37	6·1	0·5590
	1×6	60 ft. × 18 ft.	3·3	6/242	40	1,981·2	86·99	4·4	0·4029
	2×3	120 ft. × 9 ft.	13·3	6/242	40	1,981·2	107·85	5·4	0·6055
	8×1	480 ft. × 3 ft.	160	8/242	30	2,641·6	130·54	4·9	0·6342
	4×2	240 ft. × 6 ft.	40	8/242	30	2,641·6	136·55	5·2	0·6732
	4×3	240 ft. × 9 ft.	26·7	12/242	20	3,962·4	199·36	5·0	0·7924
	2×6	120 ft. × 18 ft.	6·7	12/242	20	3,962·4	147·47	3·7	0·5858
	8×2	480 ft. × 6 ft.	80	16/242	15	5,283·2	247·27	4·7	0·8600

TABLE VII

Analysis of data of tonnage experiments with Co. 213 sugarcane in 1933

Sub-block No.	Cuts × rows	Plot dimensions	Ratio of length to width	Plot acreage	Total No. of plots	Mean yield in lb. per plot	S. D. (after elimination of soil variance)	V = Coef. Var. $\left(\frac{\text{S. D.} \times 100}{\text{Mean}}\right)$	S. E. of V.
IV	1×1	60 ft. × 3 ft.	20	1/242	240	295·0	27·6	9·4	0·4375
	1×2	60 ft. × 6 ft.	10	2/242	120	590·0	49·6	8·4	0·4671
	2×1	120 ft. × 3 ft.	40	2/242	120	590·0	41·1	7·0	0·4803
	1×3	60 ft. × 9 ft.	6·7	3/242	80	885·0	76·4	8·6	0·5081
	4×1	240 ft. × 3 ft.	80	4/242	60	1,180·0	62·5	5·3	0·5682
	2×2	120 ft. × 6 ft.	20	4/242	60	1,180·0	123·2	10·4	0·5590
	1×6	60 ft. × 18 ft.	3·3	6/242	40	1,770·0	160·4	9·1	0·4929
	2×3	120 ft. × 9 ft.	13·3	6/242	40	1,770·0	117·8	6·7	0·6055
	8×1	480 ft. × 3 ft.	160	8/242	30	2,360·0	104·5	4·4	0·6342
	4×2	240 ft. × 6 ft.	40	8/242	30	2,360·0	102·4	4·3	0·6732
	4×3	240 ft. × 9 ft.	26·7	12/242	20	3,540·0	195·8	5·5	0·7924
	2×6	120 ft. × 18 ft.	6·7	12/242	20	3,540·0	275·1	7·8	0·5858
	8×2	480 ft. × 6 ft.	80	16/242	15	4,420·0	174·0	3·9	0·8600

DIFFERENCES IN YIELDS IN DIFFERENT SUB-BLOCKS

The distributions of yields of different plot-sizes in all the four sub-blocks were found generally * to be normal. The exact distributions, and the validity of Fisher's z-test as applied to these data are being tested (in a separate paper to follow). A large number of possible arrangements are taken and tested for agreement between the actual and theoretical values. In the present case, both the mean yields and the coefficients of variation were in all cases found to be very high as compared to their respective standard errors, showing a high degree of accuracy for these statistical coefficients. Comparing the mean yields of plots in the several sub-blocks, it is seen from the tables that the mean yield remains almost the same in the first three sub-blocks, while sub-block IV is found significantly inferior in fertility to the other sub-blocks. The analysis of variance of yields for the plot size 120 ft. × 18 ft. = 12/242 acre for the several sub-blocks is given in Tables VIII (a, b, c).

TABLE VIII (a)

Analysis of variance of the yields of sub-blocks (I to III) for plot size 120 ft. × 18 ft. = 12/242 acre

Variance due to	Degrees of freedom	Sum of squares	Mean square	'z'
Between blocks . .	2	137,580	68,790	0·4347
Within blocks . . .	57	1,644,123	28,844	Less than 5 per cent value of 'z' and hence not significant.
Total .	59	1,781,703	..	

TABLE VIII (b)

Analysis of variance of the yields of sub-blocks (I to IV) for plot size 120 ft. × 18 ft. = 12/242 acre

Variance due to	Degrees of freedom	Sum of squares	Mean square	'z'
Between blocks . . .	3	3,894,082	1,298,027	1.6496
Within blocks . . .	76	3,641,003	47,921	Greater than one per cent value of 'z' and hence highly significant.
Total .	79	7,535,085	..	

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* In the case of plots whose number in each sub-block is less than 20, the exact distribution is not ascertainable.

TABLE VIII (*c*)

Mean yields of sub-blocks in lbs.

Sub-blocks	I	II	III	IV	S. E. of mean of 20 plots	Critical difference (5 per cent level)
Mean yields . .	4,090	4,063	3,962	3540	48·95	137·74

(I = II = III > IV).

In the case of the first three sub-blocks, '*z*'-test shows no significant difference in yields between 'blocks', and 'within blocks' for this particular plot size, while by combining all the four sub-blocks, the variance due to 'blocks' is found to give a significant difference from that due to 'within blocks'. Comparisons between sub-blocks on the basis of '*t*'-test bring out no significant difference between the first three sub-blocks and a significant difference between either of them and the fourth sub-block. It is, therefore, considered necessary to treat the fourth sub-block as distinct from the other sub-blocks, for the purpose of this study.

SOIL VARIATION IN THE SEVERAL SUB-BLOCKS

To examine the differences in plot yields in the individual sub-blocks, percentage deviations of yields for the plot-size 1 cut × 3 rows (= 3/242 acre) from the mean yield of the sub-block have been calculated, and are given in Table IX Plots with positive and negative deviations exceeding ten per cent (*i.e.*, patchy areas) are shown in block type. In sub-block I, of the eighty plots there are only eight patchy plots, three of which occur in the first row and four in the fifth column. In sub-block II the number of patchy areas is almost the same, but it seems more homogeneous than the first sub-block. Sub-block III contains a slightly larger number of patchy areas than the first two sub-blocks. Sub-block IV is marked by an appreciable number of patches with the fifth and sixth columns containing a large number of high-yielding plots. Analysis of variance between cuts and rows for the several sub-blocks is given in the Table X. It is interesting to see that in all the sub-blocks there is no significant difference in variation between 'cuts' and 'rows'. But in sub-block I, the variations in cuts and rows are both significant, in sub-block II there is a significant variation in rows only, in sub-block III the variations in both cuts and rows are not significant, and in sub-block IV both the cuts and rows have significant variations. Besides, the figures of percentage variations in the plot-yields indicate no systematic change in fertility in the several sub-blocks, nor could any competition between the plots be detected. Any plan of lay-out under conditions of fertility in any of these sub-blocks should therefore take into account the trend of variation indicated above.

TABLE IX

Percentage deviations in plot-yields from the mean yield of sub-block (plot size 1 cut × 3 rows = 3/242 acre)

	Rows	Cuts							Total
	Sub-block I								
	—7	+6	+6	—15	—16	—11	—2	+5	—34
	—1	0	—1	—5	—23	0	+5	+3	—22
	+3	+7	+9	+8	—1	+9	+10	+8	+53
	—4	+6	+4	+1	+4	0	+3	0	+14
	+8	—1	+8	+13	+2	+3	+3	—4	+32
	+2	+1	+13	—5	—5	—1	+8	+10	+23
	—1	0	—1	+4	—8	—4	+3	+6	—1
	+3	+3	+2	—5	—8	—6	+4	+6	—1
	—6	+3	+2	—6	—14	—1	+1	—1	—22
	—6	+3	+8	—3	—19	—7	—4	—4	—32
Total .	—9	+28	+50	—13	—88	—18	+31	+29	
	Sub-block II								
	+3	+4	+12	—2	—3	—13	+4	+3	+8
	+1	+8	+9	+9	—3	..	+3	+1	+28
	—10	+2	+8	+5	—3	—5	—3	—5	—11
	..	+5	—6	—2	+5	+5	+4	+7	+18
	+2	+6	—5	+4	+7	—3	—3	—5	+3
	+1	—13	—1	..	+5	—1	+3	+9	+3
	+3	+1	—3	+6	—2	—1	—3	+5	+6
	+2	—7	—15	+3	..	—8	—16	—3	—44
	+10	—10	—4	1	—13	..	—3	+4	—16
	—5	—8	+1	—13	—9	—1	—2	+5	—32
Total .	+7	—12	—4	+10	—16	—27	—16	+21	
	Sub-block III								
	—5	—3	+1	—5	—23	—19	—9	—2	—65
	—2	—7	+4	+6	—10	—1	+3	+6	—1
	+3	—1	+5	+8	+6	+11	+4	+1	+37
	—5	—4	+2	—2	—0	+5	+7	+6	+9
	—1	—9	—13	+1	—6	—1	—3	+4	—28
	—13	+8	—1	—2	—5	—5	+5	+2	—11
	—8	+11	+3	+11	—2	—1	+7	+12	+33
	+6	—1	—6	+4	+7	+7	+10	+7	+34
	—3	—10	—4	+3	—2	+4	—1	—5	—18
	—8	—5	+3	+1	+14	+3	+4	—4	+8
Total .	—36	—21	—6	+25	—21	+1	+27	+27	
	Sub-block IV								
	—4	—9	—7	+5	+31	+15	—4	—9	+18
	—7	—8	+9	+5	+18	+4	—8	+4	+17
	+13	+3	+3	+4	+6	+19	..	+13	+61
	—1	—14	—7	+3	+12	+28	—1	+17	+37
	+5	—20	—6	+12	+15	+17	+2	+11	+36
	—13	—29	—2	+14	+9	+12	+1	—4	—12
	+5	—10	—4	+5	+10	+18	—4	—13	+7
	+1	—10	—10	..	—3	+7	—1	—8	—24
	+3	—16	—6	—7	—18	—7	+1	—1	—51
	+4	—26	—24	..	—23	—2	—10	+10	—71
Total .	+6	—129	—54	+41	+57	+111	—24	+20	

TABLE X

Analysis of Variance

[*Based on data in Table IX*]

Variance due to	Degrees of freedom	Sum of squares	Mean square	'z'
		Sub-block I		
Cuts	7	4,046	578·0	(1) 1·017 Cuts and error.
Rows	9	1,922	213·6	(2) 0·520 Rows and error.
Error	63	4,884	75·5	
Total .	79	10,852		

Variations in 'Cuts' and 'Rows' are both significant as compared to error.

Variance due to	Degrees of freedom	Sum of squares	Mean square	'z'
		Sun-block II		
Cuts	7	426	60·9	(1) 0·2933 Cuts and error.
Rows	9	1,162	129·1	(2) 0·6690 Rows and error.
Error	63	2,134	33·87	
Total .	79	3,722		

Variation in 'Cuts' is not significant as compared to error. Variation in 'Rows' is significant.

Variance due to	Degrees of freedom	Sum of squares	Mean square	'z'
		Sub-block III		
Cuts	7	182	26·0	(1) 0·3143 Cuts and error.
Rows	9	553	61·4	(2) 0·2725 Rows and error.
Error	63	2,261	35·6	
Total .	79	2,996		

Variation in 'Cuts' and 'Rows' are both not significant.

Variance due to	Degrees of freedom	Sum of squares	Mean square	'z'
		Sub-block IV		
Cuts	7	1,339	191·3	(1) 1·0441 Cuts and error.
Rows	9	962	106·9	(2) 0·7533 Rows and error.
Error	63	1,494	23·7	
Total .	79	3,795		

Variation in 'Cuts' and 'Rows' are both significant.

Explanation of Tables IV-VII

Tables IV to VII are sufficiently self-explanatory. The standard deviation for each plot size in each sub-block is worked out by Fisher's analysis of variance after elimination of *division variance (*i.e.*, variance due to soil heterogeneity) on the assumption of five plots in each division. This assumption is made with a view to eliminate soil variance, while the same number of plots for every arrangement of plot size will ensure the same number of treatments in each block, in this case assumed to be five. For convenience, the case of plot sizes whose number in each sub-block is a multiple of five, alone has been considered. The arrangement of plots in each sub-block is in all cases rectangular. The method of analysis for working out the standard deviation is illustrated below :—

Sub-block I

Plot size 120 ft. × 18 ft. = 12/242 acre.

Variance due to	D. F.	Sum of squares	Mean Square	½ log (M. S	z
Between divisions . .	3	377324·1	125774·7	5·8712	
Within divisions . .	16	516098·0	32256·1	5·1907	0·6805
Total .	19	893422·1			

Five per cent z (for $n_1 = 3$ and $n_2 = 16$) = 0·5876

'z'—test in this case shows a significant difference between 'divisions' and 'within divisions'. Standard deviation per plot is the square root of 32256·1 *i.e.* 179·6. On this basis, the coefficient of variation (*i.e.* $\frac{\text{S. D} \times 100}{\text{Mean}}$) is then calculated. The standard error of the coefficient of variation has been calculated on the basis of the well-known formula :—

$$\text{S. E. of C. V.} = \sqrt{\frac{V}{2n}}\,\frac{\surd}{r}\overline{\left[1+2\left(\frac{V}{100}\right)^{2}\right]},$$

where V is the coefficient of variation.

Table XI shows that by increasing the width or length of the plot the coefficient of variation rapidly goes down. Taking sub-blocks I—III, the series giving low coefficients of variation are :—2 rows × 8 cuts, 3 rows × 4 cuts and 6 rows × 2 cuts ; and the averages for sub-blocks I—IV, also give the same plot-sizes. But since the plot should have a sufficient number of rows to allow for border effect and convenience of cultivation, of the various plot-sizes, 6 rows × 2 cuts (*i.e.*, 12/242 acre) seems desirable.

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*In this paper, 'division' is used in the sense of common usage 'block', not to confound with 'block' and 'sub-block' already used in the paper.

TABLE XI

Average coefficient of variation for different plot-sizes and shapes

Length of cut (60 ft.)	Rows (between row to row 3 feet)			
	1	2	3	6
	(1) Sub-blocks I—III			
1	9·0	6·4	5·8	4·5
2	6·7	5·6	4·9	3·9
4	5·5	4·6	4·1	
8	4·3	3·7		
	(2) Sub-blocks I—IV			
1	9·1	6·9	6·5	5·7
2	6·8	6·8	5·4	4·9
4	5·4	4·6	4·5	
8	4·3	3·8		

TABLE XII

Average coefficient of variation from several sub-blocks for different plot-sizes

Plot size (acreage)	Sub-blocks I—III	Sub-blocks I—IV
1/242	9·0	9·1
2/242	6·4	6·9
	6·7	6·8
3/242	5·8	6·5
4/242	5·5	5·4
	5·6	6·8
6/242	4·5	5·7
	4·9	5·4
8/242	4·3	4·3
	4·6	4·6
12/242	4·1	4·5
	3·9	4·9
16/242	3·7	3·8

Table XII and Fig. 1 emphasise the same point that the coefficient of variation diminishes gradually till a plot-size 12/242 to 16/242 of an acre is reached. This decrease in the coefficient of variation with increased size of plots is consistent with observations on other crops in other countries. The average coefficient of variation for the size 16/242 acre is 3·7 for the first three sub-blocks, and 3·8 for all the four sub-blocks taken together, and for the size 12/242 of an acre the averages are 3·9 and 4·5 respectively. In the same graph, theoretical coefficients of variation for different plot-sizes are also shown, and it is clear that the reduction in the actual coefficient of variation as plot-size increases is found less than that based on theoretical considerations. This indicates the existence of a correlation between the plots, and this point is examined in a subsequent para.

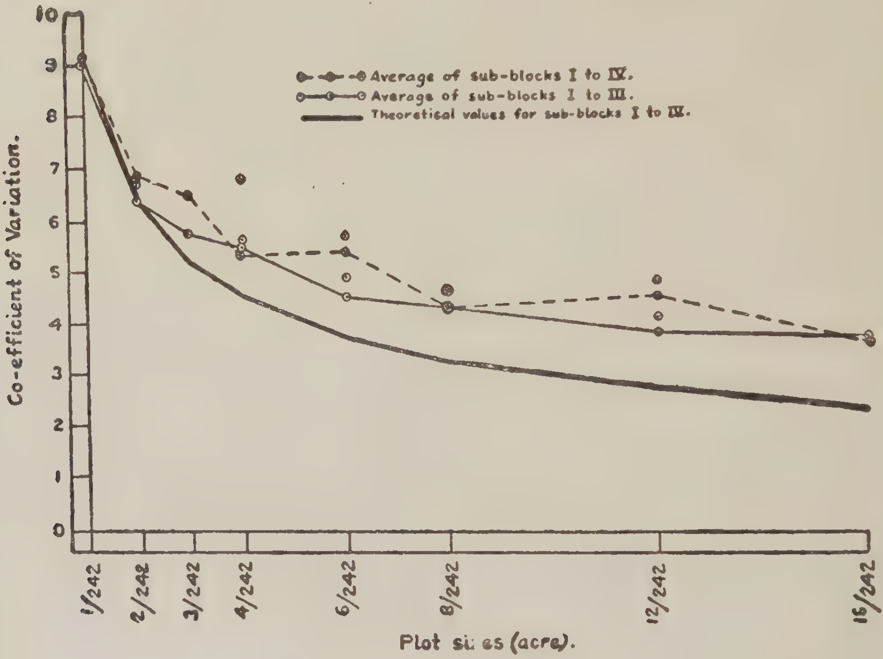


Fig. 1 (*vide* Table XII). The relation between coefficient of variation and size of plot.

(Stray points indicate different shapes for the same size.)

CHANGES IN THE COEFFICIENT OF VARIATION AS PLOT SHAPE CHANGES

Table XIII and Fig. 2 indicate how the coefficient of variation changes as the shape changes *i.e.*, as the ratio of length to width of the plot increases.

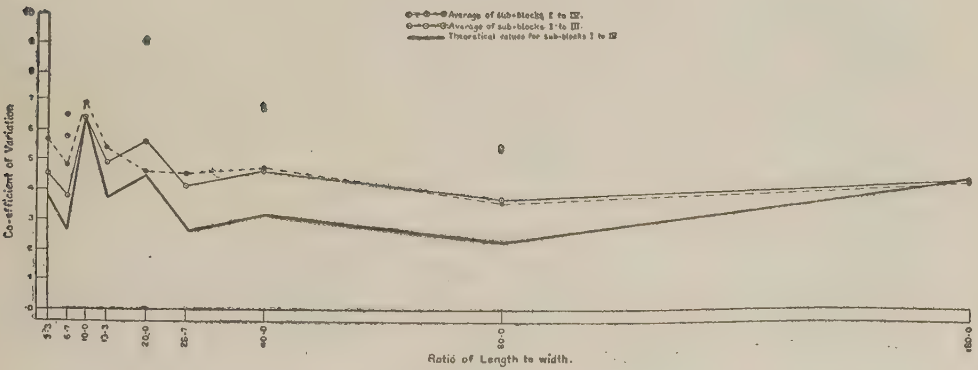


Fig. 2 (*vide* Table XIII). The relation between coefficient of variation and ratio of length to width of plot.

(Stray points indicate smaller sizes for the same shape.)

It is interesting to see that in the several sub-blocks, the coefficient of variation increases till the ratio 10 is reached ; then it shows a definite decline between the ratios 10 to 20 and thereafter has a fall, though not markedly, beyond ratio 20. The peaks at the ratios 10 and 20 in the graph are associated with very small plot-sizes. Excluding these two peaks, the smallest values centre round the ratios 6·7, 26·7 and 80. Leaving out of account the ratios 26·7 and 80 for the very good reason that the number of rows to be dealt with in such cases is very small—too small for making due allowance for border effect—the experimental plots, the ratio of whose length to width is about 7 : 1, seem to give low coefficient of variation, as compared to plots with lower ratios. The plot-size corresponding to this ratio is 12/242 of an acre. This emphasises the need for a long narrow experimental plot for sugarcane of about 12/242 of an acre in area.

TABLE XIII

Coefficient of variation for different plot shapes

Ratio of length to width	Sub-block I	Sub-block II	Sub-block III	Sub-block IV	Average for sub-blocks I—III	Average for sub-blocks I—IV
3·3 (6/242 acre) . .	4·9	4·2	4.4	9·1	4·5	5·7
6·7 (3/242 ,,) . .	6·0	5·0	6.4	8·6	5·8	6·5
6·7 (12/242 ,,) .	4·4	3·5	3.7	7·8	3.9	4·9
10·0 (2/242 ,,) . .	6·0	5·9	7·2	8·4	6·4	6·9
13·3 (6/242) . .	4·8	4·5	5·4	6·7	4·9	5·4
20·0 (1/242 ,,) . .	9·2	8·4	9·5	9·4	9·0	9·1
20·0 (4/242 ,,) .	6·0	4·7	6·1	10·4	5·6	6·8
26·7 (12/242 ,,) .	4·4	2·9	5·0	5·5	4·1	4·6
40·0 (2/242 ,,) . .	6·7	6·1	7·4	7·0	6·7	6·8
40·0 (8/242 ,,) . .	4·7	3·9	5·2	4·3	4·6	4·6
80·0 (4/242 ,,) . .	5·2	5·0	6·2	5·3	5·5	5·4
80·0 (16/242 ,,) . .	3·9	2·5	4·7	3·9	3·7	3·8
160·0 (8/242 ,,) . .	3·8	4·1	4·9	4·4	4·3	4·3

TABLE XIV

Variances for plots varying in size and shape

Length of cut 60 ft.	Rows			
	1	2	3	6
	Average of sub-blocks I—III			
1	932	1,863	3,463	8,339
2	2,021	5,738	9,845	24,230
4	5,449	15,590	28,390	
8	13,415	41,682		
	Average of sub-blocks I—IV			
1	889	2,012	4,056	12,680
2	1,963	8,098	10,853	37,099
4	5,063	14,314	30,877	
8	12,792	38,830		

RELATIVE PRECISION OF DIFFERENT PLOT SIZES AND SHAPES

We have so far compared the different plot-sizes and shapes on the basis of their coefficient of variation, that is, on the basis of their standard deviations corrected for their means or for their plot-sizes. But the relative plot-precision of one plot size to another can be judged on the basis of their variances ; thus if one plot-size gives a variance A, and another a variance B, the relative precision of the first to the second is B/A. A plot size, however, has to satisfy other considerations, such as the number of replications possible with that size in an experimental field of a given extent. Modern methods of experimentation insist upon a sufficient number of replications and upon particular modes of arrangement. Replication in an experiment secures an error as low as possible and ensures a valid estimate of it. Therefore, in choosing one plot-size to another, our object should be to choose one with not only a lower variance, but one which would permit of proportionately larger number of replications in a field of a given extent. Thus, if the first size be n times the second with reference to a field of a given extent, the former can permit of only $\frac{1}{n}$ times the number of replications which the latter can admit, the relative precision of the first plot to the second (on the basis of variances A and B respectively) corrected for the number of replications will then be Bn/A.

Table XIV gives the average variances for different plot-sizes and Table XV their relative precision, assuming the precision of the smallest plot size to be 100.

TABLE XV

Relative precision of different plot-sizes corrected for replications

Plot acreage	Relative precision Blocks I—III	Relative precision Blocks I—IV
1/242 (1 cut × 1 row)	100·0	100·0
2/242 (1 cut × 2 rows)	100·0	88·4
2/242 (2 cuts × 1 row)	92·2	90·6
3/242 (1 cut × 3 rows)	80·7	65·8
4/242 (4 cuts × 1 row)	68·4	70·2
(2 cuts × 2 rows)	65·0	41·9
6/242 (1 cut × 6 rows)	67·1	42·1
(2 cuts × 3 rows)	58·9	49·2
8/242 (8 cuts × 1 row)	55·6	55·6
(4 cuts × 2 rows)	47·8	50·0
12/242 (2 cuts × 6 rows)	46·1	28·8
(4 cuts × 3 rows)	39·4	34·6
16/242 (8 cuts × 2 rows)	36·0	36·7

It is clear that on the above criterion of efficiency the smallest plot-size is the most desirable and that the relative precision decreases from 100 to 36 as the plot-size increases from 1/242 of an acre to 16/242 of an acre. The fall in efficiency is marked till we reach 8/242 of an acre, and thereafter between 8/242 and 16/242 of an acre the fall is not so rapid. Though the smallest plot-size available is the most efficient in view of a very large number of replications which it can permit, still from the point of view of cultivation and to take account of border effect an irreducible minimum number of rows in a plot seems necessary.

Again between plots of the same size and different shapes, the differences in efficiency are not very marked, though generally speaking long narrow plots have, for all practical purposes given very good results.

DIVISION VARIANCE (*i.e.*, IN USUAL LANGUAGE BLOCK VARIANCE) *vs.* PLOT VARIANCE

We have seen from the present data that as plot-size increases, the coefficient of variation rapidly goes down. From general considerations, it is clear that with a very small plot-size, the variance between the plots is very small and that as the plot-size increases, the plot variance also increases owing to inclusion in each plot of differences in fertility obtaining in the field. Thus in any block arrangement, with small-sized plots, a larger portion of block variance is eliminated leaving a small plot variance, and with the increase in the size of plot beyond a certain stage, the elimination of block variance is not so effective, and the plot variance becomes large. If A be the block variance and B the plot variance, in fixing a suitable plot size we are seeking one after elimination of A giving as small a value as possible for B. In the case of a plot-size, where $B > A$ significantly, the lay-out fails in eliminating effectively the block variance, and the plot-size in such a case should be reduced. When B is small and A appreciable enough, the lay-out may be considered satisfactory ; for in such a case while block-elimination is successful the plots will represent a random and sporadic variation in each block. An ideal lay-out is the one where the block-size should represent a sample of the general fertility in the field, and the plot-size a random variation giving a low standard error.

In the present case, from Table XVI it is seen that as plot-size continuously increases, the block arrangement is successful in eliminating an appreciable portion of division variance. When the plot size is 1/242 acre, the percentage of the sum of squares due to divisions to total sum of squares is between twenty-eight and thirty-six per cent in the first three sub-blocks and sixty-four per cent in the fourth sub-block. As plot size increases the variance due to divisions continuously increases and so also the plot variance. In no case, however, (excepting plots of 6/242 of an acre and above in sub-block IV) is the plot variance found higher than division variance. Again, when the plot-size is between 12/242 and 16/242 of an acre there is a successful elimination of block variance in all the sub-blocks ; giving to the size of that order a definite bias. Lastly, the need for a sufficient number of replications in a field experiment is brought out ; thus a plot-size of 1/242 acre with forty-eight replications gives a higher standard of accuracy than a size of 2/242 acre with twenty-four replications. The size of 12/242 acre which we have considered to be suitable from other stand-points can be replicated only four times in each of the sub-blocks.

TABLE XVI

Plot size (Figures within brackets represent acreage)	Degrees of freedom		Total sum of squares		Variance		Whether difference in the previous columns is significant or not
	Divisions	Within divisions	Sum of squares due to divisions (Per cent of total)	Sum of squares due to within divisions (Per cent of total)	Due to divisions	Due to within divisions	
Sub-block I							
60′ × 3′ (1/242) .	47	192	36	64	2,353	989	Significant
60′ × 6′ (2/242) .	23	96	46	54	6,366	1,697	,,
120′ × 3′ (2/242) .	23	96	43	57	6,672	2,070	,,
60′ × 9′ (3/242) .	15	64	38	62	10,216	3,770	,,
240′ × 3′ (4/242) .	11	48	35	65	11,952	5,041	,,
120′ × 6′ (4/242) .	11	48	36	63	16,968	6,665	,,
60′ × 18′ (6/242) .	7	32	45	55	38,255	10,181	,,
120′ × 9′ (6/242) .	7	32	36	64	25,639	9,722	,,
480′ × 3′ (8/242) .	5	24	40	60	36,135	10,962	,,
240′ × 6′ (8/242) .	5	24	23	77	24,063	16,770	Not significant.
240′ × 9′ (12/242) .	3	16	16	84	33,175	31,791	,,
120′ × 18′ (12/142) .	3	16	42	58	1,25,775	32,256	Significant.
480′ × 6′ (16/242) .	2	12	19	81	67,504	45,284	Not significant.
Sub-block II							
60′ × 3′ (1/242) .	47	192	36	64	1,884	818	Significant.
60′ × 6′ (2/242) .	23	96	39	61	4,379	1,616	,,
120′ × 3′ (2/242) .	23	96	36	64	4,011	1,722	,,
60′ × 9′ (3/242) .	15	64	35	65	5,905	2,611	,,
240′ × 3′ (4/242) .	11	48	37	63	11,778	4,679	,,
120′ × 6′ (4/242) .	11	48	40	60	11,797	4,084	,,
60′ × 18′ (6/242) .	7	32	31	69	15,430	7,242	,,
120′ × 9′ (6/242) .	7	32	25	75	12,583	8,172	Not significant.
480′ × 3′ (8/242) .	5	24	32	68	27,266	12,254	Significant.
240′ × 6′ (8/242) .	5	24	34	66	28,235	11,342	,,
240′ × 9′ (12/242) .	3	16	33	67	36,751	13,619	,,
120′ × 18′ (12/242) .	3	16	13	87	15,999	19,881	Not significant.
480′ × 6′ (16/242) .	2	12	43	57	87,354	18,605	Significant.
Sub-block III							
60′ × 3′ (1/242) .	47	192	28	72	1,593	992	Significant.
60′ × 6′ (2/242) .	23	96	23	77	2,902	2,275	Not significant.
120′ × 3′ (2/242) .	23	96	31	69	4,580	2,372	Significant.
60′ × 9′ (3/242) .	15	64	26	74	6,231	4,007	,,
240′ × 3′ (4/242) .	11	48	29	71	11,862	6,626	,,
120′ × 6′ (4/242) .	11	48	22	78	8,195	6,464	Not significant
60′ × 18′ (6/242) .	7	32	33	67	17,328	7,569	Significant.
120′ × 9′ (6/242) .	7	32	26	74	18,860	11,642	Not significant.
480′ × 3′ (8/242) .	5	24	34	66	43,104	17,030	Significant.
240′ × 6′ (8/242) .	5	24	16	84	17,441	18,659	Not significant.
240′ × 9′ (12/242) .	3	16	7	93	17,279	39,760	,,
120′ × 18′ (12/242) .	3	16	37	63	69,108	21,756	Significant.
480 × 6′ (16/242) .	2	12	13	87	59,634	61.157	Not significant.
Sub-block IV							
60′ × 3′ (1/242) .	47	192	64	36	5,378	762	Significant.
60′ × 6′ (2/242) .	23	96	62	38	17,173	2,460	,,
120′ × 3′ (2/242) .	23	96	67	33	14,516	1,689	,,
60′ × 9′ (3/242) .	15	64	57	43	32,926	5,837	,,
240′ × 3′ (4/232) .	11	48	65	35	33,015	3,906	,,
120′ × 6′ (4/242) .	11	48	44	56	45,885	15,178	,,
60′ × 18′ (6/242) .	7	32	43	57	91,971	25,728	,,
120′ × 9′ (6/242) .	7	32	61	39	1,00,255	13,877	,,
480′ × 3′ (8/242) .	5	24	58	52	71,728	10,920	,,
240′ × 6′ (8/242) .	5	24	71	29	1,27,626	10,486	,,
240′ × 9′ (12/242) .	3	16	48	52	1,96,348	38,338	,,
120′ × 18′ (12/242) .	3	16	39	61	2,59,229	75,680	,,
480′ × 6′ (16/242) .	2	12	61	39	2,90,985	30,276	,,

BLOCK VARIATION

In all our calculations, we have tacitly assumed that each block contains five plots. The essential criterion for a suitable block size is that while it should be such that it should typify the major soil variation obtaining in the field as we pass from one point of the field to another and that the plot in each block should not be too small to lose its identity in the block in which it is placed. Table XVII gives the coefficient of variation for different division sizes in the several sub-blocks. In the first three sub-blocks, the block variability goes down till the division size is 60/242 or 80/242 of an acre, (*i.e.*, with the corresponding plot-size 12/242 or 16/242 of an acre) ; and in the fourth sub-block the variability is least when the division size is 80/242 of an acre.

But the block-size depends upon the number of treatments to be compared, and in the present case we have assumed five plots for each block. It is worth while, however, to consider the effect on the S. E. of an assumption of four, six and increasing number of plots in each block. We see, on the basis of five plots, that the block variation is small enough (as may be seen from Table XVII), and thus a comparison of five treatments (or approximately five) seems suitable under the soil conditions of the experimental field. It should be remembered, however, that the number of treatments to be compared cannot be unduly large, thereby increasing the block-size to an abnormal extent and diminishing the local control.

TABLE XVII

Coefficient of variation for different division sizes i.e., *in usual language block sizes* (i.e., $\frac{\textit{Block S.D.}}{\textit{Block Mean}} \times 100$)

Division size (acreage)	Sub-block I	Sub-block II	Sub-block III	Sub-block IV
5/242	2·8	2·6	2·4	4·9
10/242	2·3	1·9	1·6	4·4
	2·7	1·9	2·1	4·1
15/242	1·9	1·5	1·6	6·8
20/242	1·7	1·6	1·6	3·1
	1·9	1·6	1·4	3·6
30/242	1·9	1·2	1·3	3·4
	1·6	1·1	1·4	3·5
40/242	1·4	1·2	1·6	2·3
	1·1	1·2	1·0	2·9
60/242	0·9	0·9	0·7	3·5
	1·6	0·6	1·4	2·9
80/242	1·3	1·1	0·9	2·2

OTHER CONSIDERATIONS FOR FIXING A SUITABLE PLOT-SIZE

Every experimenter knows that in a field experiment with small plots, 'border effect' is a very important consideration for drawing any valid inference. The statistical criteria for testing the border effect in the case of a field trial are, firstly whether the standard error of the experiment changes in any way by the exclusion of border rows,and secondly whether the comparative effects of different treatments with the whole plots harvested are in any way changed by the exclusion of border rows from the plot. We cannot however apply both the criteria in the case of a uniformity trial, but applying the first test for the first sub-block to the plot-sizes with a minimum of three rows in the case of the present data, it is found that the coefficient of variation has increased in every case by the exclusion of border rows.

The following table gives the results :—

TABLE XVIII (*a*)

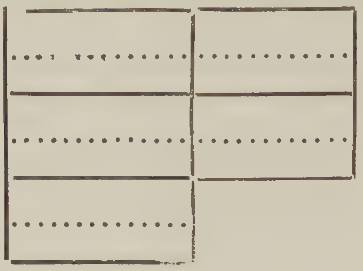
Plot-size	Coefficient of variation by harvesting the whole plot	Coefficient of variation by excluding two border rows
1 cut × 3 rows = 3/242 acre . .	6·0	9·3
1 cut × 6 rows = 6/242 acre . .	4·8	5·6
2 cuts × 3 rows = 6/242 acre . .	4·9	6·5
2 cuts × 6 rows = 12/242 acre . .	4·3	4·8
4 cuts × 3 rows = 12/242 acre . .	4·4	4·5

It is clear that in the case of the present trial, the exclusion of borders has produced a significant change in the coefficient of variation. This is in accordance with the results obtained by Immer [1932]. The question of competition has been further examined by working out correlation coefficients between the yields of any row and the mean of yields of adjacent rows in the first sub-block.

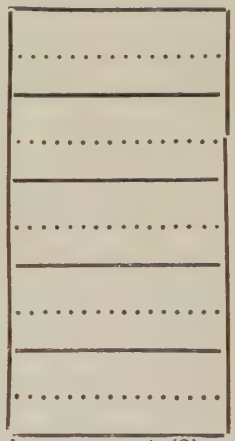
The value of correlation coefficient obtained was +0·1281 (not significant).

This shows that the competition between rows is negligible in the case of the present data ; if there should be any competition, the correlation should be negative and significant, unless the soil differences concealed any competition that might be present.

Another important consideration connected with the lay-out of an experiment is whether the mode of arrangement of plots in a block exercises any influence on the standard error of the experiment. It is a common experience that more compact a block is, the less the standard error. For example, of the two arrangements given below of the same plot-size the arrangement (1) will probably give a lower standard error than (2), as in the former case a greater proportion as 'block variance' may be removed by analysis.



Arrangement (1)



Arrangement (2)

A fundamental problem in connection with the lay-out of an experiment is whether Latin square arrangement or randomised block method is suitable. While the latter has the advantage that any number of blocks or replications can be had, the former has the advantage, in leading to an effective elimination of variances in two directions. The types of experiment however, (*i.e.*, whether it is a varietal trial, manurial or irrigation) determines to a large extent the method of lay-out to be adopted.

From the present data of the uniformity trial, Table XVIII (*b*) has been calculated for a few cases only, to see which of the two methods of lay-out gives a low coefficient of variation. The figures generally show that except in cases of randomised block arrangement where an adequate number of replications is adopted (say five or six), the Latin square arrangement is found more efficient than the randomised block arrangement.

*The data further show that the decrease in the coefficient of variation with the increase in the number of replications (as the basis of the same number of plots in each block) is not consistent with theoretical expectations. Thus one would expect the coefficient of variation of five varieties with six replications to be $\sqrt{\frac{3}{6}}$ times the coefficient of variation of five varieties with three replications; in the present case it is much greater than what may be expected. This may be accounted for by the fact that the proportion of block variance eliminated in the analysis of variance is not always consistent with the number of replications adopted, but depends to a considerable extent upon the variation in the blocks themselves.

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*Thanks are due to Mr. Yates, Chief Statistician, Rothamsted, for having brought this point to the notice of the authors.

TABLE XVIII (*b*)

Sub-block I

Average coefficient of variation for different types of lay-out

Plot-size	Latin square type			Randomised block arrangement			
	3 × 3	4 × 4	5 × 5	5 varieties × 3 replications	5 varieties × 4 replications	5 varieties × 5 replications	5 varieties × 6 replications
2 cuts × 3 rows = 6/242 acre	5·0	4·8	4·6	5·6	5·2	5·1	4·8
4 cuts × 3 rows = 12/242 acre	4·0	3·9		4·4	4·1		
8 cuts × 2 rows = 16/242 acre	3·7						

TABLE XIX

Theoretical number of replications to bring down the C. V. to two per cent

Length of cut (60 ft.)	Rows (between row to row 3 feet)			
	1	2	3	6
		Sub-blocks I—III		
1	20	10	8	5
2	11	8	6	4
4	7	5	4	
8	4	3		
		Sub-blocks I—IV		
1	20	12	10	8
2	12	12	7	6
4	7	5	5	
8	4	4		

THEORETICAL NUMBER OF REPLICATIONS NECESSARY TO BRING THE C. V. TO TWO PER CENT.

Assuming that the standard deviation per plot for different plot-sizes which we have deduced in Tables IV–VII are those which could be normally expected, the question is how many replications are necessary to bring the coefficient of variation to a low level say to two per cent.

The object of replications being to secure a low error, the number of replications necessary for each plot-size should be examined to see whether we could afford sufficient land consistent with the theoretical number of replications required.

Table XIX gives the theoretical number of replications necessary to bring down the coefficient of variation to two per cent on the basis of the data given in Table XI. For example, coefficient of variation for two rows and four cuts is 4·6 from Table XI; to bring it down to two per cent on this basis, the number of necessary replications is obtained from the formula : $\frac{4\cdot6}{\sqrt{n}}=2$, *i.e.* $\sqrt{n}=2\cdot3$ or $n=5\cdot3$.

Thus five replications of the particular plot-size will be necessaryto reduce the coefficient of variation to two per cent. With a coefficient of variation of two percent in a single mean, the coefficient of variation of the difference of two means is $2\sqrt{2}$ per cent and the difference in two means necessary for significance will be $2\sqrt{2} \times 2$, *i.e.*, 5·66 per cent ; and the number of replications given in Table XIX will be sufficient to bring down the error in the case of each plot-size to this level of significance. In the case of size of two cuts × six rows, (*i.e.*, 120 ft. × 18 ft. = 12/242 acre) the number of replications required will be four on this basis.

TABLE XX

Extent of land necessary for different plot-sizes to answer the theoretical number of replications (the nearest integer being taken in each case from Table XIX) and assumption of five plots in each block

Length of cut (60 ft.)	Rows			
	1	2	3	6
	Average of sub-blocks I—III (in $\frac{1}{242}$ acres)			
1	100	100	120	150
2	110	160	180	240
4	140	200	240	
8	160	240		
	Average of sub-blocks I—IV (in $\frac{1}{242}$ acres)			
1	100	120	100	240
2	120	240	210	360
3	140	200	300	
8	160	320		

EXTENT OF LAND NECESSARY BASED ON THEORETICAL NUMBER OF REPLICATIONS

Table XX shows the extent of land necessary for different plot-sizes on the basis of the theoretical number of replications given in Table XIX and on an assumption of five plots in each block. Even if the number of plots in each block changes, the ratios of the extents of area as shown in Table XX will remain the same. Taking the size 2 cuts × 6 rows, the number of plots in each block five, and the number of replications four (to bring down the C.V. to two per cent), the extent of land necessary is 12/242 × 5 × 4 = 240/242 acre ; while for a size 2 cuts × 3 rows the extent of land necessary will be 6/242 × 5 × 6 = 180/242 acre ; and so on. It is thus seen that on the basis of results of the first three sub-blocks, from the point of view of economy in land, of the one-cut plots, 1 cut × 1 row or 1 cut

× 2 rows is the most economical ; of two-cut plots 2 cuts × 1 row is most economical and so on. But we have already seen that from other considerations six-row plots are suitable and that more than one cut to bring down the S. E. a plot-size of 6 rows × 2 cuts, (*i.e.*, 12/242 acre) demands an extent of 240/242 acre seems suitable. But costs of cultivation, planting and harvesting are not always proportional to the areas cultivated in view of some constan t expenditure involved in cultivation. It is therefore necessary that in case when between two or three plot-sizes one has to be decided upon not only is the extent of land the main consideration but also costs of cultivation involved.

Comparison of work in other countries with different crops

Though the size of the experimental plot for severa l crops under different conditions has been engaging the agricultural experim enters in other countries, so far no special attention has been paid to the shape of the plot. The question of shape has been studied by Christidis [1931] and his conclusions are :—

(1) In agricultural experiments it seems that significant results cannot be secured by only using appropriate statistical methods ; uniformity amongst the individual plots is more essential than anything else.

(2) In order to test the validity of the assumptions made regarding the effect of the shape of the plots, the numerical data of several uniformity trials have been considered (on mangolds, potatoes, oats, and green forage). A close agreement was found between expectation and actual results in the great majority of cases, the evidence being remarkably significant in favour of the long plots. In only three cases were the results inconclusive, this apparently being accounted for by the way in which the original plots were formed, causing an inequality in area amongst them.

(3) In the light of these investigations, in order to reduce the effect of soil heterogeneity, the plots used should be as long and narrow as possible. This, of course within the limits set by different practical considerations, amongst which convenience, competition (when acting), and the accurate measurement of the width appear to be the most important.

These conclusions tally remarkably with our own inferences on the data of the present experiments on un-irrigated sugarcane.

Summary and conclusions

1. The need for standardising a suitable size and shape for an experimental plot for all crops in different farms in India grown under different conditions is very urgent ; this question is assuming a definite importance in the case of sugarcane which is now one of the most important economic crops in India. It is further important to recognise the need for a correct statistical technique to be applied to data from experiments conducted for the purpose of deducing a suitable size and shape for an experimental plot.

2. An experiment was conducted on unirrigated sugarcane (Co. 205) in Harpur Jhilli in 1932, with a view to deduce a suitable size and shape for the experimental plot. The yield figures for 840 plots of plot-size 30 ft.-3 in. × 3 ft. were available. The coefficient of variation was worked out for each plot-size and though broad conclusions were possible from these data, it was found that a high variation in fertility between plot and plot and row and row was a disturbing factor, and it was therefore thought advisable to repeat the experiment in 1933 on a more popular variety of cane than Co. 205 and on a soil of more uniform fertility.

3. The 1933 experiment was conducted at Meghaul (Monghyr District) on Co. 213 cane. This variety was bred by Dr. C. A. Barber at Coimbatore. Its high value in the mill trial, its remarkable tonnage capacity, and its agricultural habit on an estate scale were first tested by the Secretary, Sugar Bureau, at present the Imperial Agriculturist, Pusa. It now occupies the first place as regards area under cultivation in Bihar, Bengal and the United Provinces. Its average tonnage in the Pusa Farm is twenty-two tons per acre.

4. The land under the present experiment was under regular sugarcane rotation in previous years ; cultural methods, manuring and other operations were uniform all over the area. The lay-out of the experiment consisted of, after removing the border crop, two blocks of sizes 480 ft. × 315 ft. and 480 ft. × 93 ft. In the first block there were 105 rows and in the second 31 rows—the rows being separated by three feet. Each row was cut in eight equal sections and the ultimate plot thus measured 60 ft. × 3 ft. = 1/242 acre. The field was divided into four sub-blocks, each measuirng nearly one acre, for the purpose of this study (which necessitated the omission of a few rows).

5. Analysis of variance in yields has been worked out for : (*a*) blocks and (*b*) within blocks for the first three sub-blocks and also for all the four sub-blocks. Coefficient of variation V and its standard error, have been calculated for each plot-size in the several sub-blocks.

6. It is found that the variation in fertility between the first three sub-blocks is not marked, and that the fourth sub-block is found definitely inferior in fertility to the other sub-blocks.

7. All the values of coefficient of variation are found to be high as compared to their standard error, showing their reliability.

8. Based upon trend in the values of coefficient of variation a plot-size between 12/242 acre and 16/242 acre seems desirable.

9. The shape of the plot has to be decided not only on the basis of trend in the coefficient of variation, but also from the point of view of convenience of cultivation, and an irreducible minimum number of rows to avoid border effect ; based upon these standpoints the ratio of length to width of the experimental plot is found to be at least 6·7. Long narrow plots are thus found to sample the soil best,

and this tallies with the conclusions reached by Christidis, published in *Journal of Agricultural Science*, January 1931, (pages 14—37).

10. From the point of view of efficiency, based upon standard error relative to size, the smallest plot-size is the most efficient, but there are other considerations, such as convenience in cultivation and reduction of border effect, that decide a suitable plot-size. In the present experiment, it is seen that between plots of the same shape, efficiency has not appreciably changed provided the plot is sufficiently narrow. The present data also emphasise the fact that between two plot-sizes one of which is to be preferred, the smaller size with a greater number of replications is more efficient than the larger size with a lesser number of replications.

11. Analysing the variances due to ' divisions ' and ' within divisions ' in the case of each plot-size, two points are made clear :—(1) The block demarcation in a field trial is essential and (2) in the case of the present experiment, a plot-size between 12/242 of an acre and 16/242 of an acre eliminates an appreciable portion of soil variance.

12. Examining other considerations for fixing a suitable plot-size, in the case of the present data the border effect is found significant but competition between rows is negligible.

13. Again it is found that between ' Latin square ' and ' randomised block arrangement ' the former is found more efficient than the latter, except when the latter is provided with a sufficient number of replications, say five or six.

14. Theoretical number of replications to bring down the coefficient of variation to two per cent has been calculated in the case of each plot-size, and it is found that in the case of 2 cuts × 6 rows (*i.e.*, 12/242 acre), the number of replications required is four.

15. Extent of land necessary on the basis of theoretical number of replications, and on the basis of five plots in each block, has been calculated in the case of each plot-size. It is found that a plot-size of 12/242 acre requires 240/242 of an acre for the whole experiment. Costs of cultivation are also an important consideration in the case of a field experiment, which should be worked out for different plot-sizes.

16. Considered from all stand-points on the basis of the data in the present experiment, a plot-size of 12/242 of an acre (120 ft. × 18 ft.) seems eminently suitable for a field experiment on un-irrigated sugarcane.

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Appendix

TONNAGE EXPERIMENT WITH Co. 213, 1933

Each row is 3 ft. apart and the cuts are 60 ft. Area of each section is 180 sq. feet

(Yield in pounds)

Row No.	A	B	C	D	E	F	G	H
Block I								
1	389·0	382.0	360·0	306·5	264·0	321·0	354·0	360·5
2	302·0	369·0	415·5	320·0	293·0	293·0	344·0	376·5
3	261·0	328·0	306·0	239·5	305·0	295·5	303·5	334·0
4	337·0	310·0	319·5	367·5	288·0	344·0	313·5	330·0
5	314·5	341·0	342·5	322·0	265·0	331·5	416·0	335·0
6	356·0	371·0	345·0	280·0	242·5	350·5	342·5	389·0
7	384·0	353·5	368·0	303·0	322·0	363·5	372·0	363·5
8	330·0	377·5	402·0	445·5	313·5	366·5	360·5	334·5
9	338·0	362·5	340·0	356·0	376·0	380·0	392·0	407·0
10	311·5	346·0	345·5	343·5	334·5	339·0	393·5	396·5
11	346·5	335·0	389·0	321·5	357·0	353·5	333·0	315·5
12	323·0	402·5	324·5	372·0	372·5	334·0	327·0	305·5
13	351·5	314·0	375·0	420·0	392·0	374·5	337·0	308·5
14	402·5	333·5	351·0	405·5	327·5	311·5	321·5	332·0
15	346·0	364·0	377·0	333·0	326·0	366·5	392·0	339·5
16	393·5	303·5	363·0	326·0	338·5	352·5	413·0	413·0
17	320·0	387·0	402·5	342·5	320·5	299·0	306·0	357·0
18	328·0	337·5	387·5	305·0	311·0	360·5	389·5	359·5
19	336·0	368·0	302·0	400·0	325·0	354·5	341·5	376·0
20	327·0	335·0	365·0	332·0	270·0	269·0	338·0	350·0
21	352·5	322·5	344·0	330·5	242·0	361·0	371·5	355·5
22	358·0	352·5	325·0	349·0	315·0	350·0	389·0	370·0
23	357·0	330·0	343·5	319·5	315·0	305·0	347·0	355·0
24	337·5	366·5	371·0	299·5	310·5	305·0	323·5	359·0
25	312·5	339·5	348·0	324·0	309·0	283·0	339·0	308·0
26	362·0	306·0	338·0	331·0	259·5	358·5	355·5	340·0
27	290·0	412·0	357·5	305·5	312·0	367·0	334·0	365·0
28	318·0	363·5	349·5	354·5	257·0	289·0	314·0	347·0
29	315·5	353·0	398·5	348·5	286·0	350·0	332·0	290·0
30	325·0	337·0	359·0	289·0	287·5	316·5	339·0	345·0
31	354·0	360·0	387·0	338·0	350·5	281·0	368·0	348·0
32	340·0	330·5	370·0	342·0	321·5	314·5	339·5	370·0
33	355·0	370·0	393·5	319·5	317·5	290·5	348·5	330·0
34	373·0	393·0	333·5	376·0	328·5	335·5	385·0	355·0
35	314·0	353·0	388·5	378·5	364·0	310·0	331·5	330·0
36	341·0	351·0	385·0	358·0	293·0	369·5	327·5	341·5
37	295·0	362·0	387·0	363·0	315·0	319·5	320·0	322·0
38	328·0	355·0	351·0	358·0	343·5	334·0	327·5	338·0
39	295·0	324·0	364·5	351·0	322·0	316·0	336·0	305·0
40	307·0	364·0	379·0	323·0	316·0	345·0	331·0	335·0
41	339·0	340·0	301·5	326·5	394·5	361·5	402·0	369·5

Appendix—*contd.*

Row No.	A	B	C	D	E	F	G	H
42	365·0	360·0	275·5	351·0	357·0	363·0	328·0	380·0
43	360·0	363·0	348·5	357·0	336·0	366·0	279·5	352·0
44	372·0	346·5	360·0	343·0	388·5	360·5	407·0	327·0
45	304·0	369·0	261·0	360·0	359·0	260·0	295·5	382·0
46	332·0	289·0	312·5	289·5	346·0	362·0	317·0	380·0
47	317·0	285·0	348·5	343·5	372·0	304·5	321·0	347·5
48	380·0	305·0	341·5	381·0	352·0	339·0	410·0	380·0
49	366·5	350·5	332·5	365·0	330·0	326·0	350·0	347·5
50	357·5	340·0	299·0	350·0	350·0	336·0	316·0	330·5
51	325·0	338·5	349·5	361·5	319·0	343·0	318·0	385·0
52	391·0	324·0	311·5	331·0	322·5	298·0	250·0	357·0
53	313·5	315·0	286·5	331·0	350·0	311·5	272·0	333·5
54	337·0	308.0	264·0	385·0	341·5	322·5	334·0	299·0
55	326·0	305.0	301·0	310·0	242·0	305·5	321·5	312·5
56	393·0	319·5	311·5	361·0	308·0	359·0	342·5	365·5
57	395·0	294·5	359·0	342·0	339·0	354·0	410·0	378·5
58	230·0	288·0	321·0	284·0	313·5	377·5	293·0	333·5
59	363·0	330·0	371·0	298·5	304·5	323·0	374·0	354·0
60	375·0	317·0	333·0	306·0	309·5	305·0	330·5	382·5
61	347·5	358·0	356·0	307·0	295·0	282·0	283·0	342·0
62	312·0	271·0	318·0	305·0	313·0	254·0	325·0	311·0
63	277·0	327·5	326·5	330·0	158·0	267·0	295·0	320·0
64	275·5	300·0	340·0	364·0	427·0	308·0	365·0	371·0
65	378·0	345·0	340·5	364·0	315·0	322·0	342·0	347·0
66	315·0	280·0	346·0	322·0	361·0	335·0	316·0	335·0
67	329·0	327·0	315·0	347·0	354·5	378·0	349·0	350·5
68	333·0	340·0	313·5	301·5	337·0	350·0	338·0	338·5
69	356·0	323·0	413·0	422·0	353·5	368·0	342·0	314·5
70	333·0	303·0	328·5	376·0	315·0	336·0	359·0	384·5
71	321·5	337·0	339·5	297·0	335·0	377·5	356·0	349·0
72	291·5	310·0	345·0	295·0	339·0	331·0	344·0	311·5
73	315·5	320·5	340·0	333·5	337·0	357·5	301·0	365·0
74	348·5	300·0	332·5	345·0	327·5	315·5	340·0	345·0
75	318·0	286·0	292·5	317·5	270·0	307·5	317·5	318·5
76	269·5	327·0	285·0	316·5	324·5	286·0	355·0	315·0
77	276·0	365·0	388·0	308·0	307·0	348·0	362·0	356·5
78	316·5	378·0	310·0	345·0	305·0	308·5	318·5	333·5
79	337·5	362·0	334·0	342·0	323·0	305·0	350·0	372·0
80	266·5	366·0	322·5	330·5	267·0	335·0	335·0	309·5
81	312·0	374·0	361·5	425·0	383·0	340·0	370·0	425·0
82	319·0	403·5	334·0	335·5	326·0	367·0	401·0	367·0
83	400·0	308·5	284·5	357·5	355·0	354·0	320·0	341·0
84	328·5	270·0	315·5	341·0	375·0	340·0	370·0	356·0
85	325·0	288·5	332·0	374·0	325·5	349·5	338·0	295·0
86	321·0	325·0	312·0	343·0	300·0	345·0	306·5	326·0
87	315·0	280·0	305·0	308·0	343·0	333·0	332·5	316·0
88	278·0	333·0	338·0	332·0	386·0	322·0	340·0	317·5
89	309·0	286·0	341·0	328·5	385·0	369·5	370·0	313·5
90	326·0	325·0	343·5	339·0	362·0	327·5	321·5	316·5
91	314·5	288·0	330·0	306·0	356·5	331·0	305·0	272·5

Appendix—*contd.*

Row No.	A	B	C	D	E	F	G	H
92	320·0	343·5	340·0	286·0	286·0	274·5	290·0	285·0
93	336·0	315·5	313·0	313·5	328·0	295·0	320·0	312·5
94	333·0	315·0	258·0	308·0	296·5	310·0	315·5	327·5
95	345·0	347·0	342·0	328·0	359·0	312·0	332·0	321·0
96	354·0	303·0	287·5	269·0	361·0	323·5	342·0	360·0
97	345·0	262·5	293·0	322·5	325·0	314·0	370·5	356·5
98	322·5	272·0	298·5	356·0	327·0	373·0	375·5	351·0
99	293·0	261·0	206·0	295·0	271·5	330·0	358·0	343·0
100	313·5	289·5	274·5	336·5	368·5	331·5	333·5	294·5
101	291·5	265·0	242·5	321·0	317·5	367·5	333·5	301·0
102	331·0	271·0	279·0	298·5	342·0	313·0	371·5	283·5
103	351·5	290·0	309·5	340·5	273·5	315·0	310·0	329·5
104	315·0	266·5	335·0	315·5	310·0	340·0	319·5	324·5
105	276·5	232·5	285·0	285·0	330·0	316·0	352·0	320·0
				Block III				
106	276·0	285·0	279·5	319·5	397·5	341·5	324·0	253·0
107	280·0	252·0	243·0	328·5	395·0	330·0	245·0	275·0
108	297·5	268·5	303·0	280·5	373·5	348·5	284·5	278·0
109	279·5	285·0	324·0	343·0	357·5	330·0	290·0	293·0
110	332·5	285·0	325·0	333·5	372·0	303·5	258·5	290·0
111	212·5	242·0	315·0	255·0	318·5	287·0	268·0	336·0
112	356·0	340·0	335·0	277·5	318·0	356·0	311·5	306·5
113	330·0	292·0	314·5	342·5	296·0	350·5	300·0	335·5
114	315·0	277·0	259·5	300·0	327·5	346·5	276·0	362·0
115	300·0	280·0	310·0	288·0	331·0	411·0	307·5	332·0
116	263·0	256·5	294·0	319·0	335·5	391·0	265·5	392·5
117	310·5	228·0	215·0	308·0	322·5	335·0	304·5	311·0
118	343·5	214·0	280·0	328·5	302·0	317·0	305·0	345·5
119	256·0	236·5	260·0	294·0	337·5	342·0	306·5	297·0
120	328·0	257·0	292·5	367·5	383·0	379·0	295·5	343·5
121	270·0	216·0	306·5	325·0	323·0	328·0	304·5	306·5
122	266·5	199·0	262·0	340·5	330·0	321·0	330·0	312·0
123	231·0	207·0	294·5	343·0	312·0	339·0	262·5	229·0
124	287·5	234·5	258·5	347·0	338·0	350·0	280·0	250·0
125	294·0	261·5	295·5	277·0	312·0	329·5	300·5	267·0
126	262·5	303·0	293·0	304·5	323·5	370·0	273·5	250·0
127	307·0	279·5	304·5	285·5	310·0	350·0	255·5	288·5
128	264·5	253·5	233·0	290·0	284·0	285·0	302·0	258·5
129	319·5	262·0	259·5	306·0	264·0	315·0	316·0	264·5
130	315·0	245·5	271·0	269·0	251·0	263·5	297·5	276·0
131	280·0	236·5	293·0	250·0	228·5	274·0	291·0	295·5
132	313·5	265·0	266·0	300·0	250·0	287·5	310·0	303·0
133	291·5	197·0	230·0	298·0	225·5	280·0	233·0	374·0
134	293·5	229·0	226·0	277·0	232·0	290·0	261·0	307·5
135	339·5	225·0	216·0	304·0	220·0	297·5	303·0	290·0
136	248·5	210·0	205·5	281·0	290·0	301·0	239·5	285·0

THE BASIS FOR MERGING *SCLEROSPORA INDICA* WITH *SCL. PHILIPPINENSIS*

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(With Plate XLII)

Further study of the genus Sclerospora reveals the necessity for revising the status of certain species. In a previous paper Weston and Uppal [1932] presented the basis for establishing Kulkarni's variety *Andropogonis sorghi* of *Sclerospora graminicola* as a distinct species *Sclerospora sorghi*. In the present paper the status of *Sclerospora indica* Butler and of *Sclerospora philippinensis* Weston is considered, since recent comparative studies have revealed that the differences between these two are not sufficient to justify maintaining them as separate species.

Sclerospora indica, when first reported on maize from Pusa by Butler [1913], was regarded by him as identical with the maize mildew described as *Peronospora maydis* by Raciborski [1897] from Java in 1897. Butler, however, pointed out that the fungus was clearly a *Sclerospora* rather than *Peronospora* and hence designated it as *Sclerospora maydis* (Rac.) Butler. Later work by Palm [1918] corroborated Butler's conclusion that the Javan mildew was indeed a *Sclerospora* but showed that it was specifically distinct from the Indian mildew ; hence in 1931 Butler and Bisby [1931] very properly re-named the latter *Sclerospora indica.*

Sclerospora philippinensis, when described from the Philippines as a new species by Weston [1920] in 1920, had already previously been reported from the Philippines in 1916 by Baker [1916], who first called attention to its destructiveness to maize, and in 1918 by Reinking [1918], who first described and illustrated the structural characters of the fungus.

Both Baker and Reinking assumed that the Philippine fungus was identical with that reported from India by Butler, the general impression at the time being that the destructive downy mildew of maize in the Orient was a single entity first reported from Java, later found in India, then in the Philippines and probably already established and awaiting recognition in other countries as well.

In 1920, however, when Weston was confronted with the question of the identity of the Philippine maize mildew, the situation had become more complex. Palm had shown that the species in Java was distinct from that in India, whilst in Australia D. S. North had encountered a similar, although distinct, mildew destructive to sugarcane and had called the attention of Lyon [1915] to Miyake's little known report [1911] describing this species as *Scl. sacchari* and noting its destructiveness to maize as well as sugarcane in Formosa. Moreover Weston, during his investigation of the Philippine mildew, having found that the production of conidiophores and conidia in the field normally occurred at night when the host plants were covered with dew, had worked out the structural characters in detail and from comparisons with dried herbarium material, had concluded that an adequate concept of the distinguishing diagnostic features could be gained only from material collected at the time of optimum production and studied either when fresh or when suitably killed and preserved. At that time, the only material of the Javan, Indian and Formosan species available for comparison consisted of inadequate dried herbarium specimens, which even when supplemented by the published descriptions hardly gave adequate basis for decision even though indicating that the Philippine fungus was apparently distinct from the Javan and Formosan species, and probably from the Indian species as well.

Confronted with these difficulties Weston [1920] concluded that the best way to meet the situation was to regard the Philippine fungus as a separate species and emphasised (p. 119) " the need of comparative studies using optimum material " to settle the question of identity conclusively.

In recent years the writers have been given the opportunity of making such a comparison of *Sclerospora philippinensis* with *Scl. indica.* Uppal [1930, 1931, 1932], in the course of his investigations into various *Sclerosporas* at Poona, has studied carefully preserved material of *Scl. indica* from Pusa kindly furnished to him by Dr. W. McRae, and has been increasingly impressed by its agreement with *Scl. philippinensis.* The living material * of the maize *Sclerospora*, collected at the time of optimum production in the field at Pusa, has also been studied. Recently Weston has compared excellently preserved material of the Indian fungus from Pusa with his own Philippine material already described [Weston, 1921, 1923] and has corroborated Uppal's conclusion as to their agreement.

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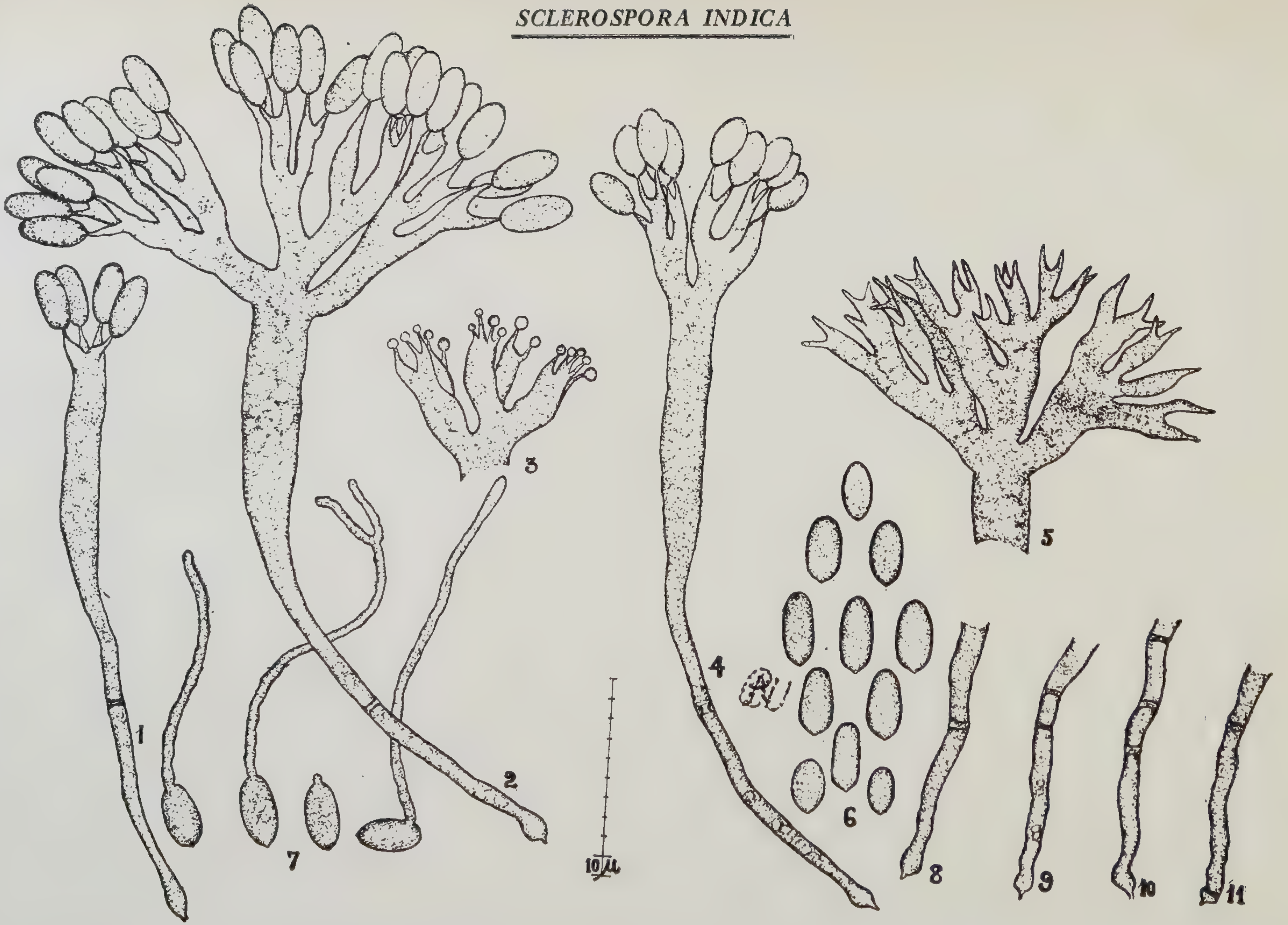
* Mr. M. K. Desai assisted the writers in making measurements of the living material of *Sclerospora indica* collected under conditions of optimum production at Pusa, Bihar.

SCLEROSPORA INDICA



1. A slender conidiophore which has failed to branch and has terminated in conidia directly. 2. A well-developed, mature conidiophore with conidia. 3. Portion of a conidiophore showing conidia just budding out. 4. A slender, sparingly branched conidiophore bearing few conidia. It is partially mature as can be seen from the rotund shape of conidia and from the incomplete development of the basal septum. 5. Portion of a profusely branched conidiophore after the conidia have been detached from the sterigmata. 6. Conidia showing the range of variation in shape and size. 7. Conidia showing direct germination by hyphae. 8, 9 and 10. Bases of conidiophores showing one, two and three septa, respectively. 11. Base

These comparative studies of the optimum material conclusively show that the slight differences between the Indian and the Philippine maize mildews do not justify maintaining these two as separate species. The agreement between these two fungi holds in all their essential features.

The conidiophores are, of course, of the well-developed type characteristic in general of the conidial *Sclerosporas* of the Orient with a differentiated basal cell (Plate XLII, figs. 1, 4, 8-11), stout main axis (Plate XLII, figs. 1, 2, 4), and extensive spreading branch system (Plate XLII, figs. 2-5), comprising stout, usually dichotomous branches terminating in the tapering sterigmata which bear the spores.

These features are well shown in Plate XLII included here to supplement Butler's [1913] original illustrations which unfortunately were based on incomplete specimens poorly developed and lacking the characteristic basal cell. A comparison of the present figures with those of Weston's [1920, 24-25] earlier illustrations of the Philippine material shows close agreement in all essential structural features. Furthermore, the dimensions are in close agreement : the total length of the conidiophores in both ranges from 150 to 410μ, most commonly from 300 to 350μ, the greatest diameter of the main axis in both is most commonly from 20 to 25μ, and the basal cell is typically about 80 to 100μ in length, and 9 to 11μ in diameter. Even the sterigmata, which were emphasised by Weston [1920, p. 117] as a point of difference, are now found to be more closely in agreement, since they vary greatly in length from as little as 6μ to as much as 30μ but average around 14 to 15μ.

The conidia, which have been found to be the most reliable basis of comparison in the species of *Sclerospora*, here show close agreement both in shape and in size. In shape they are most commonly elongate ellipsoid, elongate ovoid, or rotund cylindric (Plate XLII, fig. 6 and Weston's [1920] Plates 24 and 25). In size, although there are slight variations in the modes of length and of width, there is nevertheless a distinct overlapping in the length frequencies, sixty per cent of the Indian material falling in the range of 33 to 41μ, and sixty-five per cent of the Philippine material in the range of 31 to 39μ. In width, however, the frequencies overlap over almost the entire range of spore measurements, although the conidia of *Scl. indica* are slightly narrower (Table I). As has been suggested by Dr. Butler in a recent letter, these slight variations in conidial measurements may be regarded as characteristic of a geographical race ; for it has been his experience in comparing different samples of material received at the Imperial Mycological Institute from collections in widely separated localities that such material may show substantial differences in spore measurements even though belonging to the same species of fungus causing the same disease in the same host. Such differences in size, therefore, unless very pronounced, with no intermediates and augmented with additional structural distinctions, do not seem to justify recognition of the two mildews as separate species.

TABLE I

Comparison of measurements of conidia of Scl. indica *and* Scl. philippinensis

Length			Width		
Classes in μ	Number of conidia in 400		Classes in μ	Number of conidia in 400	
	**Scl. indica*	** *Scl. philippinensis*		*Scl. indica*	*Scl. philippinensis*
17 to 19	0	1	11 to 13	5	1
19 to 21	0	1	13 to 15	40	8
21 to 23	3	2	15 to 17	135	41
23 to 25	3	1	17 to 19	97	160
25 to 27	3	4	19 to 21	53	148
27 to 29	4	10	21 to 23	51	41
29 to 31	11	35	23 to 25	14	1
31 to 33	24	68	25 to 27	5	0
33 to 35	42	75			
35 to 37	46	64			
37 to 39	79	55			
39 to 41	75	30			
41 to 43	36	24			
43 to 45	31	21			
45 to 47	23	7			
47 to 49	9	2			
49 to 51	5	0			
51 to 53	3	1			
53 to 55	1	0			
55 to 57	2	0			

* Conidial material collected at the time of optimum production (4 a.m.) at Pusa and measured immediately by M. K. Desai.

** Conidia collected by Weston in the Philippines in dew during optimum conidium production and measured immediately as reported in *J. Agric. Res.* **19**, No. 3, 1920 (Table I).

As a result, when all these points are considered there does not seem to be any adequate basis for maintaining *Sclerospora philippinensis* Weston of the Philippines and *Sclerospora indica* Butler found only in Bihar in India, as separate species. It is most regrettable that this is the case, for this *Sclerospora* should, in all justice, give recognition, in the authority for its name, to Butler who first called attention to it in 1913. Yet the early confusion in identity with the Javan fungus and the unfortunate taxonomic tangle which followed Palm's failure to observe the International Rules of Nomenclature in re-naming Raciborski's *Peronospora maydis* forced Butler to re-name his species *Sclerospora indica* in 1931. As a result now that our comparative study reveals no adequate basis for maintaining *Sclerospora indica* and *Scl. philippinensis* as separate species, the former must be merged with the latter under the name *Sclerospora philippinensis* Weston.

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THE TOLERANCE OF WHEAT PLANTS FOR CHLORIDES OF CERTAIN NON-ESSENTIAL ELEMENTS*

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(Received for publication on 10th January 1936)

(With eight text-figures)

INTRODUCTION

The growing interest in the problems of plant nutrition within recent years has resulted to a certain extent in the realisation of the possible relation existing between the nature and amounts of elements absorbed and the elaboration of the several organic substances at different stages of growth. Such experiments conducted under natural and controlled conditions of experimentation have led to the view that growth is mainly dependent upon a number of the so-called essential and non-essential elements. While the former are required in definite quantitative proportion, the presence of the latter in minute quantities is considered to be necessary for the proper development of plants.

The investigations of Mazé [1914 ; 1915 ; 1919], McHargue [1919 ; 1922 ; 1925 ; 1927], Warington [1923 ; 1926], Brenchley [1914 ; 1, 2], Brenchley and Thornton [1925], Sommer and Lipman [1926], and Haas and Reed [1927], besides a host of other workers, have shown how the growth of their experimental plants varied in magnitude according to the presence or absence of some of these non-essential elements and in certain cases stress has been laid on the indispensability of these elements to the growth of plants. Such a relation between growth and availability of these non-essential elements, however, remains not well understood specially with respect to the tropical and sub-tropical crops and other characteristics of these regions. The need for a systematic investigation in this direction is the justification for the present contribution.

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*(Contribution from the Institute of Agricultural Research, Benares Hindu University.)

To this end, wheat plants are raised in Knop's solution under varying concentrations of the supply of these elements and the subsequent variations in certain aspects of the morphology, growth and composition of the plants are studied with respect to shoot and root lengths, leaf area, dry matter formation and the final elaboration of certain organic substances such as the various carbohydrates as well as the organic and inorganic nitrogen. To further facilitate the analysis of the relative efficiency of the many non-essential elements under investigation their chlorides are added to the culture solution in suitable molar concentrations. The experiments are performed under rigidly controlled conditions of other conditioning variables, such that limitations in these directions may not interfere in the deduction of any genuine conclusion with respect to the influence of these elements on plant growth.

INVESTIGATION

Pure strain seeds of *Triticum vulgare* (*var.* Pusa 4) of as uniform a weight and size as possible are selected, sterilised with mercuric chloride (1 : 1,000) and then washed well with distilled water. They are then germinated on mosquito nettings moistened with Knop's solution, under controlled environmental conditions. When the seedlings have grown to the extent of approximately 6 cm., they are further selected with regard to their uniformity in size and vigour and are then transferred to wide-mouth culture jars of hard resistant glass, 500 c.c. in capacity. The glass containers are covered with black paper and are fitted with perforated corks thoroughly impregnated with molten wax. The vessels together with corks are sterilised before the addition of the culture solution and only two plants are allowed to grow therein.

Three series of experiments have been conducted. In the first, the plants are grown exclusively in Knop's solution throughout their life-cycle and designated as the control, while in the other two, seedlings raised in Knop's solution for a week, are transferred to this very solution containing the chlorides of cobalt, iodine, zinc, aluminium, manganese, lithium and sodium. Four concentrations of each chloride, namely 0·01, 0·005, 0·0005 and 0·0001M, are tried, out of which the first two belong to the second series and the last two, to the third. Such a wide range of concentrations has been specially utilised to study the effect of the different elements when supplied both in strong and weak doses and thereby to discover the optimal range of concentrations which may lead to useful stimulating results.

The plants are grown in the water culture laboratory where other environmental factors are kept under rigid control. The nutrient solution is changed twice a week and aerated every day. A free circulation of air is maintained in the laboratory in order to supply fresh oxygen and carbon dioxide to the plants throughout the entire series of experimentation.

At intervals of a week, a number of plants are taken out from each set and washed free of nutrients. The external moisture is carefully wiped off before the fresh weight of the entire plant, as well as such measurements as root and shoot lengths, leaf area and other morphological characteristics, are recorded. The plant material is then fixed in five per cent formaldehyde, washed well with distilled water and then incubated to constant dry weight in a steam oven regulated at 100°C.

The materials for the estimation of the various carbohydrates (glucose, sucrose and starch including the petosans and other acid-soluble carbohydrates) are prepared after the method of Link and Tottingham [1923] and then estimated according to Official and Tentative Methods of Analysis [1930]. The total nitrogen including the nitrogen of nitrates is obtained by the modified Kjeldahl's method [1930].

EXPERIMENTAL FINDINGS

Morphological variabilities.—The morphological variations attendant upon the addition of different chlorides in varying concentrations to the culture solution range from a stage of moderate acceleration to that of complete toxicity. In case of certain chlorides in the second series, both the concentrations ($0 \cdot 01$ and $0 \cdot 005M$ solutions) result in an immediate injury, whereas in others, the higher concentration ($0 \cdot 01M$) is severely toxic to the plants. The injury caused by the application of the lower concentration ($0 \cdot 005M$) is, however, mild and not so well pronounced as in the higher one. To the first group belongs the most characteristic case of the cobalt chloride, the toxic nature of which in $0 \cdot 01M$ concentration is evinced soon after thirty-six hours of its addition. The leaves change their normal colour from green to bluish dark and later to greenish purple. The normal turgidity is lost and the leaves wilt off badly. In four or five days the entire plant population is severely affected culminating in their death. The lower concentration, although producing more or less similar morbid symptoms, is not so toxic as to cause immediate injury. The plants so treated survive longer for a period of a week or so.

In case of iodine chloride, on the other hand, similar toxicity is evinced in both the concentrations, but the injury is manifest after a few days of the growth of plants in this solution. The roots assume a reddish tinge and cease to grow, the leaves wilt and droop down and ultimately the entire plant is killed. In the case of higher concentration ($0 \cdot 01M$) these symptoms appear as early as four days after the transfer and the plants do not survive for more than a week.

Zinc chloride, on the other hand, takes a still longer time to manifest its toxic effect which is more pronounced in case of higher concentration ($0 \cdot 01M$). About a week after the plants have been transferred to this solution, a few leaves begin to show signs of chlorosis and by the end of another week the foliage almost dries up, while the leaves that are left, remain yellow and completely chlorotic. Thus, two weeks later, in case of the higher concentration and three weeks in the lower one, almost all the plants are killed by the toxic nature of the salt—which is well in accord with the observations of Conner [1920].

It is thus seen that the plants grown in culture solution with different chlorides of 0·01 or 0·005*M* strength are soon affected by their toxic action finally culminating in their death. There are yet other chlorides which, though detrimental for plant growth, allow the plants to continue their growth and complete their life-cycle when added to culture solution in 0·005*M* strength. This has been shown to be specially the case with aluminium chloride. In this particular case, although most of the plants are killed four weeks after the transfer in 0·01*M* concentration, some of the plants in 0·005*M* solution do survive the toxic effect and ear earliest of all the cultures of the second series. The toxic nature of this salt too, however, is manifest soon after its addition to the nutrient media, by the cessation of root development followed by the loss of its normal colour. Afterwards the roots get slimy to touch and gradually begin to disintegrate. The shoot part is affected only after three weeks, when partial chlorosis and reduction in the rate of shoot elongation is noticed. The internodes remain underdeveloped, exhibiting a stunted growth of the plants. The leaves are reduced in size and later on, begin to dry off and ultimately the entire plant is killed. Such toxic effects of aluminium in high concentrations have also been observed by Yamano [1905] in case of wheat and rye and by Miyake [1916] in case of rice.

Manganese chloride, unlike aluminium chloride, exhibits no injury to the roots whereas harmful after-effects are to be noted in the shoot part after a fortnight (Table I). The after-effect is just of the reverse order when compared with the aluminium injury in as much as the growth of the roots continues throughout the life-cycle of the plant while the shoot portion shows retardation in its growth rate soon after the transfer. Four weeks later elongation of the shoot part is almost checked and the new leaves appear to arise from the same node, giving a more or less rosette appearance. The leaves are reduced in length and become chlorotic later on. Chlorosis and drying out of the leaves is a common feature as has been noticed by McCool [1913], McGeorge [1923], Rippel [1923] and others. By the end of the fifth week, the entire population of plants wilt and dry under the higher concentration. The severity is minimised to a certain extent in case of the lower concentration where, in spite of the death of a large number of plants by the end of the sixth week, the remaining ones are in a position to complete their life-cycle although without flowering. Relatively speaking the shoot is more elongated while the roots, though developed to a great extent, do not increase in length beyond that noted for the higher concentration.

The fact thus becomes evident that the addition of the different chlorides under higher concentration is decidedly more injurious than the lower one. This is further supported by the observations recorded in case of the lower concentration of lithium chloride which brings about a more satisfactory and vigorous growth of plants than is observed in the higher one (0·01*M*).

The case of sodium chloride is, however, different from those described above. The lower concentration in this case brings about a poor growth both of the shoot and the root, while the higher one surpasses even the control (Table I) as regards the general growth and development of plants—a result which is in keeping with the observations of Lipman, Davis and West [1926].

In the third series of experiment the chlorides are supplied in very low concentrations of 0·0005 and 0·0001M strength. It is to be noted, however, that whereas the lower concentration (0.0001 M) is better in some respects, the relatively higher one (0·0005M) brings about unfavourable variations in certain cases so far as the general morphology is concerned. Cobalt chloride in 0·0005M concentration, though not exhibiting leaf injury and colourations characteristic of still higher concentrations, brings about malformation in roots and stunted growth of shoot, culminating in the death of plants in the third week. On the other hand, plants growing in 0.0001M solution survive the toxic effect and grow to ear, although the setting of grains is not well marked. The degree of toxicity of cobalt chloride, thus, goes on diminishing with its dilution in the nutrient media and although concentrations lower than 0·0001M have not been tested experimentally, it is probable that still lower concentrations of this salt may exert stimulative effect, as stressed by Mazé [1915] and Nakamura [1904] for certain crops.

In case of iodine chloride solution of 0·0005M strength the root growth is practically checked. The leaves after the first week begin to show signs of severe chlorosis and drying off—the drying proceeding from the tip downward towards the sheath. Wilting is noted in the third week. In still dilute solution of 0·0001M strength, the plants survive for a longer time, but the growth of root is poor while the shoot, though exhibiting vigorous growth in the beginning, shows a slight chlorosis of leaves after about a month. With further advance in age, toxicity is more pronounced and small streaks of white patches scattered all over the surface—more towards the margin, less towards the midrib—appear in this lower concentration. Iodine, thus, even in such a dilute concentration as 0·0001M exerts its toxic effect in contrast to the observations of Aso and Suzuki [1904-05] and Mazé [1915 ; 1919] made upon other crop plants. Brenchley [1924], on the other hand, observed that different species of plants vary in their reaction towards this element. She found that iodine badly checked the germination and growth of barley and mustard, the former being more sensitive to its toxic action ; but that if some time elapsed between the treatment and the sowing, much of the toxic action of iodine disappeared with the result that the former was affected to some extent while the latter remained unaffected, suggesting thereby that much of the iodine during this interval was removed, in some way or other, from the field of action of the plant root possibly by adsoprtion by the various constituents of the soil.

Unlike the undesirable symptoms produced under high concentration, zinc chloride in 0·0005M strength leads to stimulated growth of root, shoot and assimilating surface and earlier formation of ears. It exhibits its stimulating effect even under still lower concentration (0·0001M) (Table I) as observed by Nakamura

[1904] in certain cases. The higher concentration ($0 \cdot 0005M$) is, however, favourable more for shoot growth than for the root growth as compared to the lower one.

Aluminium chloride of $0 \cdot 0005M$ strength exhibits poor development of root although the shoot growth has been encouraging. But still lower concentration ($0 \cdot 0001M$) leads to vigorous growth of root, shoot and assimilating surface (Table I) a result quite in accord with the observations of Kratzmann [1914]. In general the dilute solution is far more beneficial than the stronger ones [McLean and Gilbert, 1928]. This effect is also apparent in case of manganese chloride where increase in root and shoot length and assimilating area over the control is characteristically noted in the $0 \cdot 0001M$ concentration. That manganese in smaller doses brings about stimulative effects, has been noticed also by Loew and Sawa [1902], Aso [1907], McHargue [1922], Samuel and Piper [1929] and others.

Lithium and sodium chlorides are found to have entirely different effects inasmuch as the relatively higher concentrations are better for plant growth. Although the range of concentration of lithium chloride best suited for plant growth is between $0 \cdot 0005M$ and $0 \cdot 005M$ strength, below and above which growth being seriously affected (Table I), no remarkable increase in growth over the control has been observed by the application of this salt—a result found in contrast to the observation of Nakamura [1904]. In case of sodium chloride, however, it is to be remarked that the stimulative effect increases as the concentration is raised higher and higher, better growth being observed under $0 \cdot 01M$ concentration as indicated above. Lipman, Davis and West [1926] working on the tolerance of plants to sodium chloride have noted more or less similar response inasmuch as the decreasing concentrations of this salt resulted in the decreasing growth of the root and shoot.

Dry weight production in plants.—Like morphological variations, the dry weight of plants also, at successive stages of plant growth, undergoes modification in response to the concentrations of the different salts used for the experimental purpose. The toxic or useful effects of the different chlorides, supplied in different concentrations, soon influence the production of dry matter and lead to greater divergence in the ascending portion of the time-weight curves (Figs. 2-8), although the initial capital (dry weight) with which the plant starts its life-cycle remains practically the same in different cases (Table I).

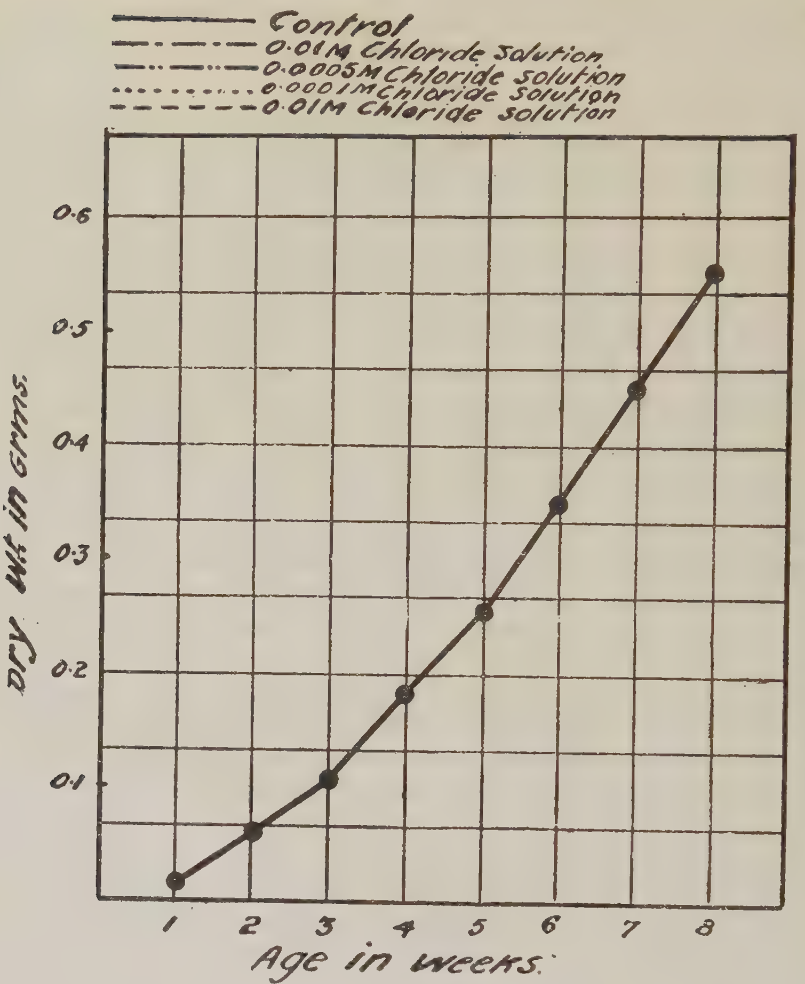
Control
0·01M Chloride solution
0·0005M Chloride solution
0·0001M Chloride solution
0·01M Chloride solution
Dry Wt in Grms.
0·6
0·5
0·4
0·3
0·2
0·1
1
2
3
4
5
6
7
8
Age in weeks.

Fig. 1.

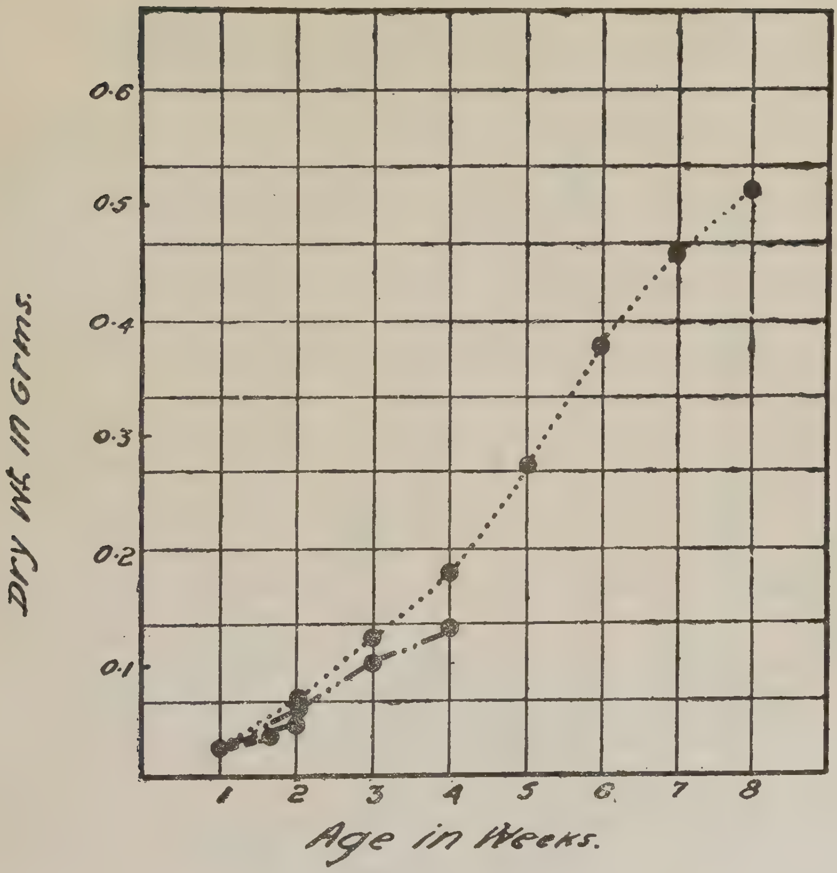

Fig. 2.

[*Please refer to the legend of Fig. 1 (p. 726).*]

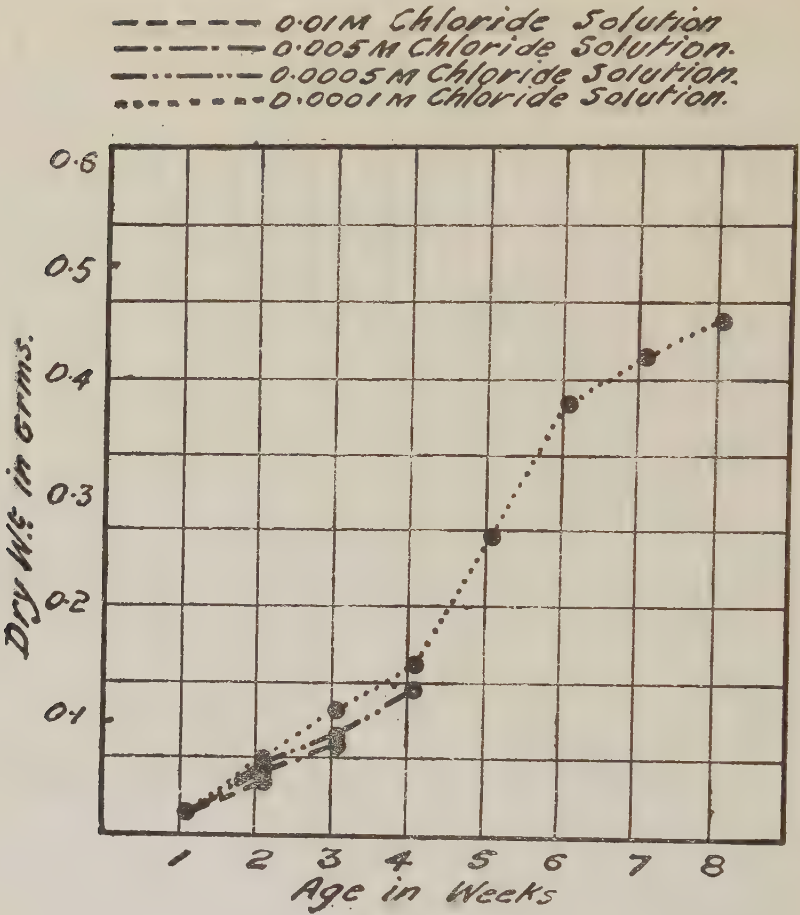
0·01M Chloride Solution
0·005M Chloride Solution.
0·0005M Chloride Solution.
0·0001M Chloride Solution.
Dry Wt. in Grms.
0·6
0·5
0·4
0·3
0·2
0·1
1
2
3
4
5
6
7
8
Age in Weeks

Fig. 3.

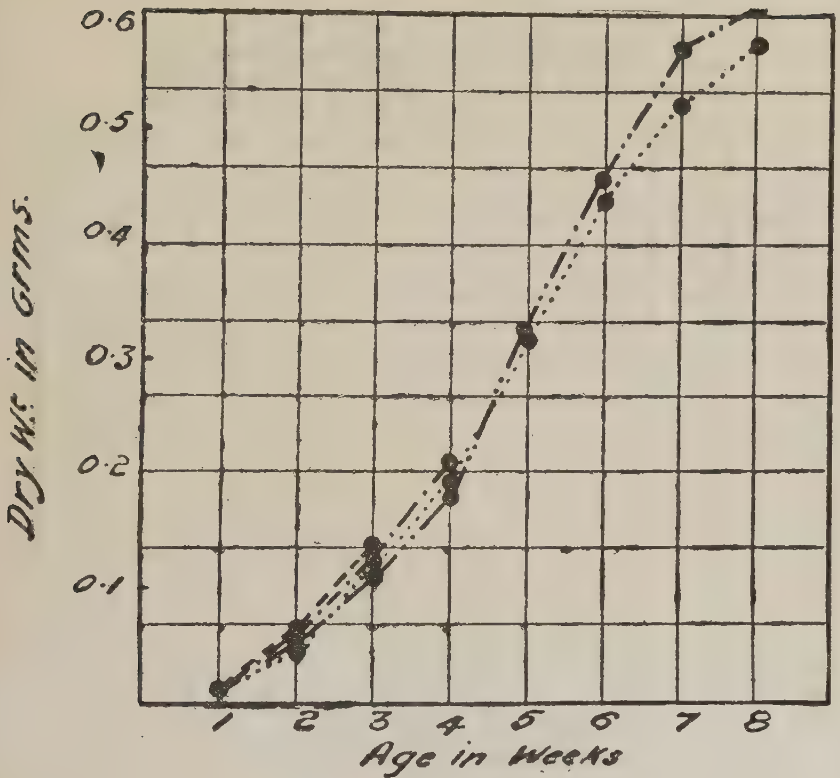

Fig. 4.

[*Please refer to the legend of Fig. 3 (p 728)*].

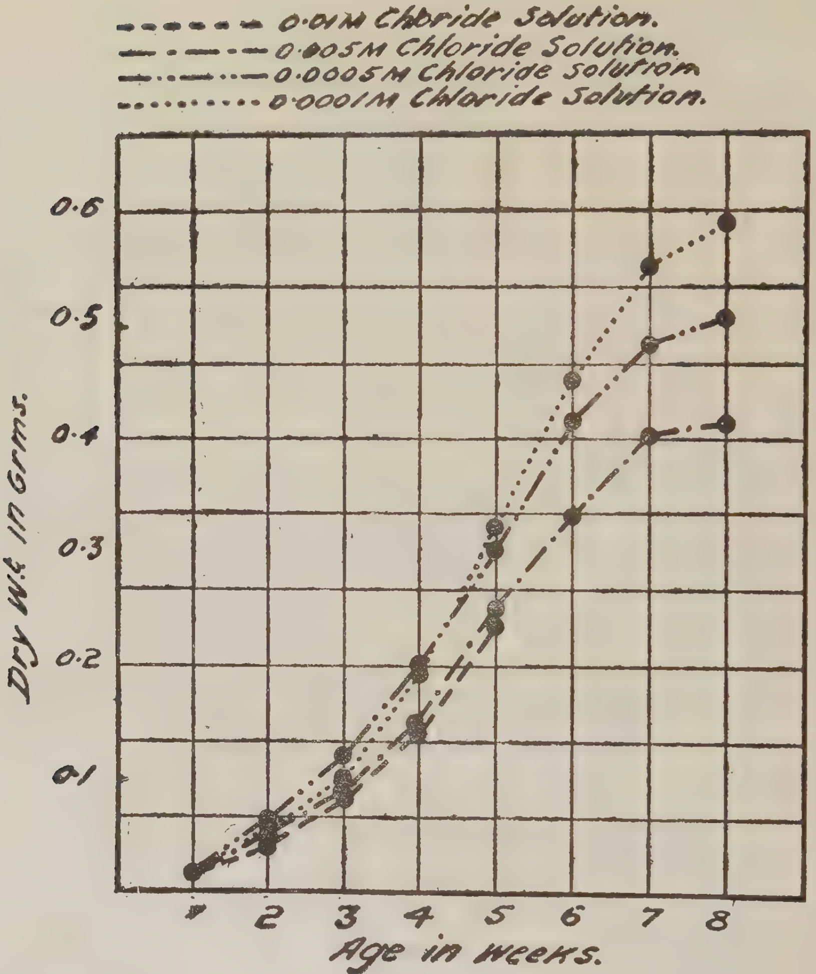
0·01M Chloride Solution.
0·005M Chloride Solution.
0·0005M Chloride Solution.
0·0001M Chloride Solution.
Dry Wt in Grms.
0·6
0·5
0·4
0·3
0·2
0·1
1
2
3
4
5
6
7
8
Age in weeks.

Fig. 5.

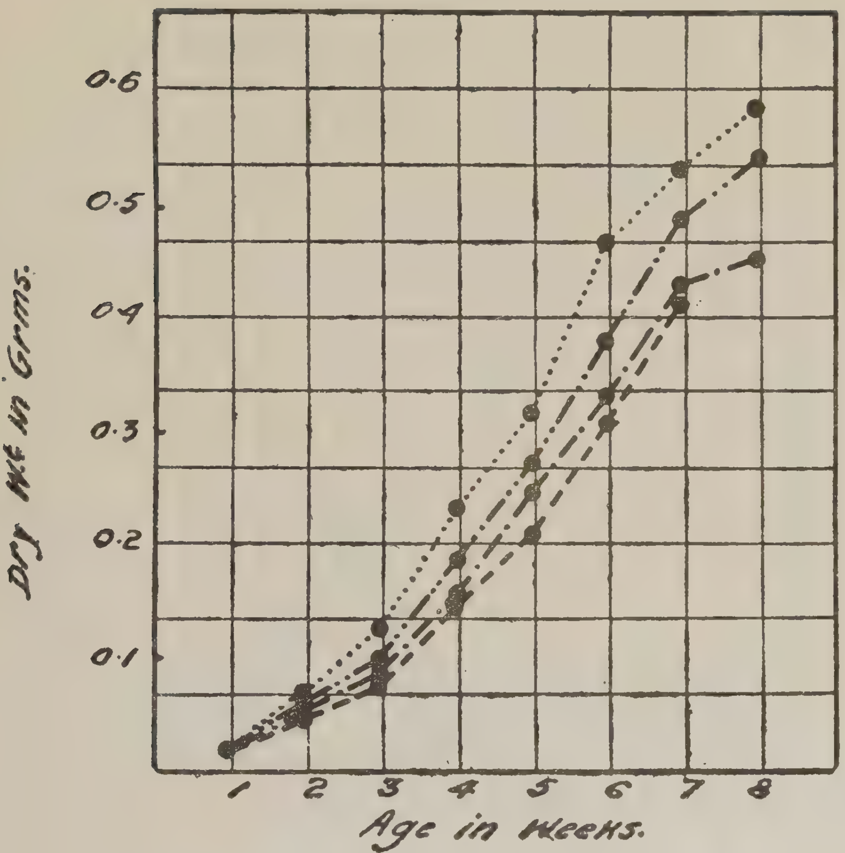

Fig. 6.

[*Please refer to the legend of Fig. 5 (p. 730).*]

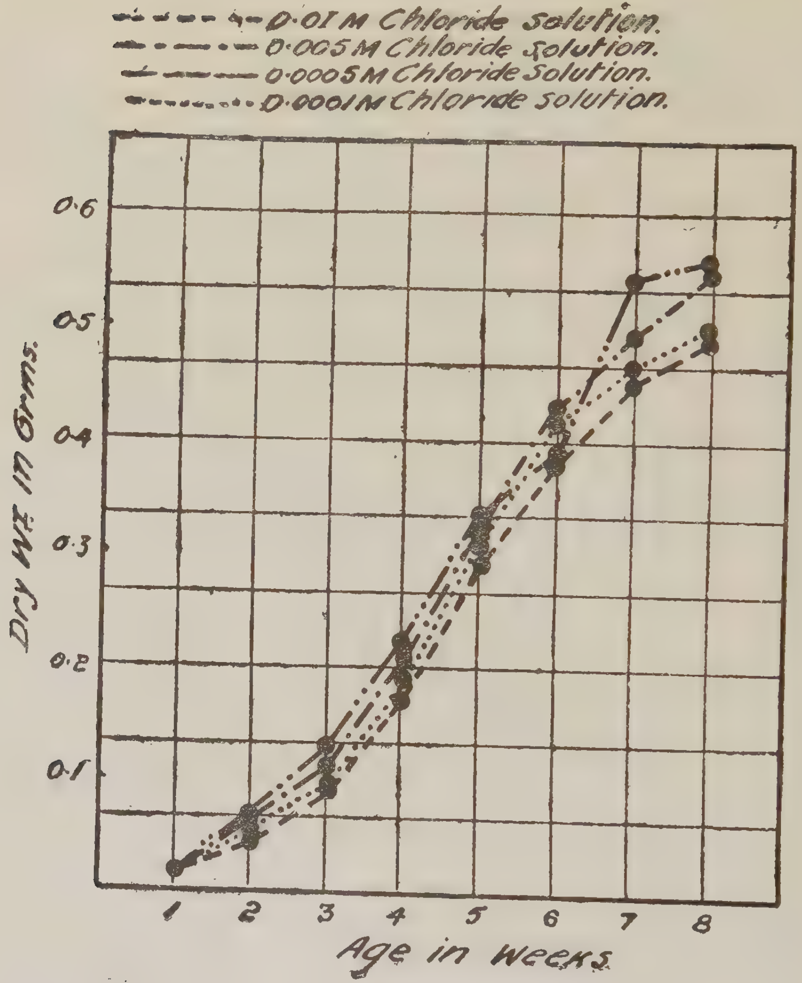
0·01 M Chloride solution.
0·005 M Chloride solution.
0·0005 M Chloride Solution.
0·0001 M Chloride solution.
0·6
0·5
0·4
0·3
0·2
0·1
Dry Wt. in Grms.
1
2
3
4
5
6
7
8
Age in weeks.

Fig. 7.

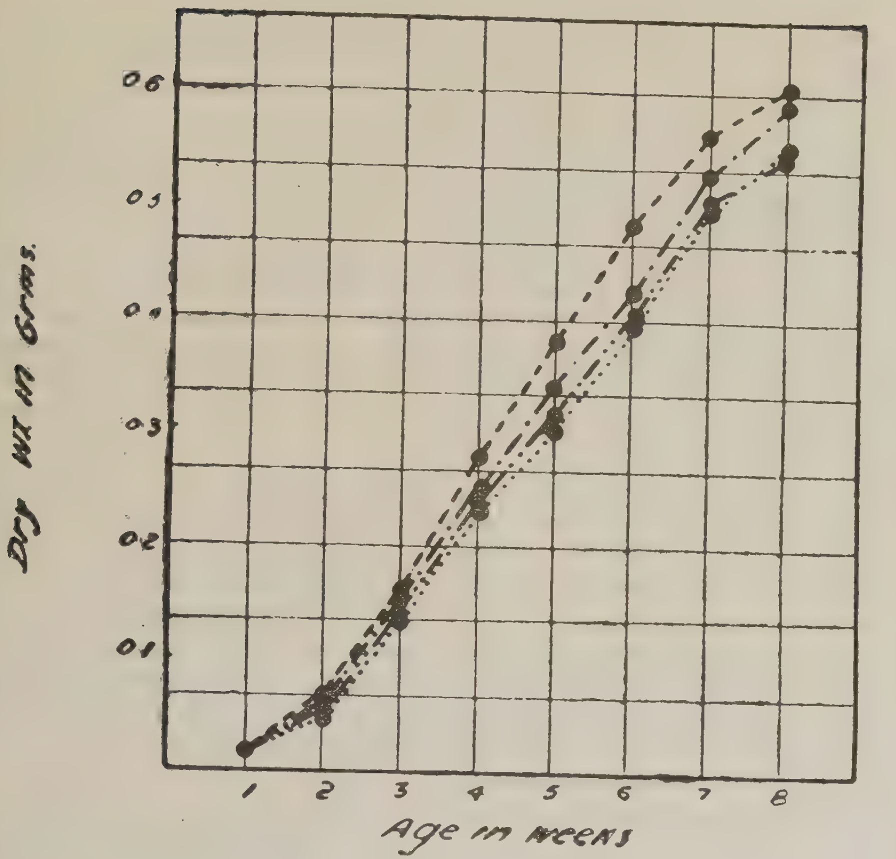

Fig. 8.

[*Please refer to the legend of Fig. 7 (p. 732).*]

While such a divergence in plants belonging to the different sets is characteristically noted in some of the salts like aluminium, manganese and zinc chlorides ; in still others like iodine, cobalt, sodium and lithium chlorides, the differentiation is not so well marked. It is to be further noted that in cases of iodine and cobalt chlorides the plants die at an early stage due to the apparently toxic effects of these salts. Especially is this true of the higher concentrations where this toxicity should naturally be more as compared to the lower ones in which case the plants are in a position to thrive better and continue to grow more vigorously for a major portion of their life-cycle. In almost all the cases, however, where the curves are complete the essential sigmoid nature is preserved (Figs. 2-8), in spite of the fact that variations have been caused in the constitution of the culture solutions. The nature of the growth reaction, thus, at successive stages remains more or less identical, but differences in intensity and magnitude of the same are to be noticed in response to the presence of the different chlorides, in the culture solutions under higher or lower concentrations.

The plants belonging to any one set although growing under differential nutritional supply do not show very significant differences in their dry weights at successive weekly stages during the early period (Table I) while with increase in the period of growth, the differences in a way become more marked at least, in certain cases where divergence in the nature of the curves has already been indicated to take place. This is all the more apparent when we take into consideration that towards the adolescent stage of plant growth photosynthentic activity markedly rises [Singh and Lal, 1935] and as such the more or less insignificant effect of these salts on the production of dry matter during early stages, becomes more significant as the age-cycle is increased.

Towards the close of the life-cycle it is to be noted that the plants growing in the nutrient media exhibit varying degrees of dry weight accumulation (Table I). In view of the observations thus collected it may be remarked that some of the culture solutions are decidedly useful in inducing greater dry matter yield whereas others exhibit only harmful effects leading to decreased dry matter formation towards the close of the life-cycle. Thus sodium, zinc, aluminium and manganese chlorides when supplied in 0·01 and 0·005, 0·0005 and 0·0001, 0·0001, and 0·0001*M* concentrations respectively induce greater dry matter yield which ranges between 0·5840 grm. to 0·6130 grm. in different cases (Table I) as compared to the control possessing 0·5480 grm. dry weight. At concentrations other than these the above four chlorides exhibit decidedly toxic influence inasmuch as the age-cycle is cut short as well as the dry matter yield is diminished. In contrast to these iodine and cobalt chlorides seem to have greater toxic effects and as such in none of the concentrations used, does the dry matter yield become equivalent to that obtained in the control plants, even though in certain cases the age-cycle remains the same. In lithium chloride, however, the dry matter yield under 0·0005*M* concentration is almost equal to that of the control.

The verdict of these statements leads us, therefore, to the view that only a few of these non-essential elements available in very dilute concentrations influence dry matter formation to some extent and as such they may be said, in general, to have very little significance of practical utility, since their presence in very small quantities in the seed or soil may be sufficient to stimulate the formation of various organic and inorganic constituents determining plant growth. To test how far the synthesis of various organic material is influenced by the availability of these elements the final chemical analysis of the plants growing in different culture solutions has been carried out with respect to the various carbohydrates (glucose, sucrose and starch) and nitrogen.

In majority of these cases the percentage of glucose does not reach to that extent as obtained in the control plants (Table II). In case of sodium chloride alone the percentage of glucose under $0{\cdot}01M$ reaches a slightly higher value than that of the control. Lithium, aluminium and manganese chlorides under $0{\cdot}0005$, $0{\cdot}0005$ and $0{\cdot}0001$, and $0{\cdot}0001M$ respectively are capable of bringing the glucose content of plants very nearly equal to that of the control. Other chlorides, however, are decidedly injurious in this direction.

As regards the sucrose content, in none of the experimental sets it is of the same order as in that of the control plants, although in certain cases, higher or lower values approaching this limit are obtained. Starch on the other hand, behaves in an entirely different manner. In majority of the sets the starch content of plants is higher than that of the control. This substance is thus capable of accumulation to a greater extent in presence of these chlorides which may thus be said to have served directly or indirectly in the increased synthesis of starch or its greater accumulation in plants.

The total carbohydrates, however, show no greater rise than the control plants in the different series of experiments except under $0{\cdot}01M$ sodium chloride, $0{\cdot}005M$ lithium chloride, $0{\cdot}0005M$ zinc chloride, and $0{\cdot}0001M$ manganese chloride. These concentrations of the above four chlorides appear to have greater stimulative effect than their other concentrations. In the cases of other chlorides the different concentrations experimented upon, appear to be toxic in their effect, bringing about a decline in the carbohydrate content of the plant tissues.

The total nitrogen, too, undergoes similar response inasmuch as, in majority of the cases its content never reaches the limit attained by the control plants except in $0{\cdot}01M$ and $0{\cdot}005M$ of zinc chloride and $0{\cdot}0001M$ of aluminium chloride.

It may be remarked here that in discussing the data on the chemical analysis of plant material the economic aspect has mainly been taken into consideration as examined by the values obtained towards the close of the life-cycle. It is, however, necessary to point out at this stage that the age-cycle in different solutions has never been a common feature throughout the entire series of experimentation. Curiously enough in certain cases, at least, the carbohydrate and nitrogen contents of the plant tissues reach the same order in the short-lived plant as that obtained for long-lived ones and sometimes surpass the latter in this respect. It thus appears that with the shortening in the age-cycle the synthesis and accumulation of these materials start at a slightly greater rate to compensate the deficiency and to bring the plants into one level of organic and inorganic content. The entire series of chemical changes occurring in the long-lived plants thus attain similar magnitude within a shorter range, in the short-lived ones. From a physiological point of view, therefore, the rate of their formation may be greater, but from an economic point of view the final outturn never appears to be paying and as such it cannot be claimed that the majority of these elements have stimulative effect upon the chemical changes attendant upon growth, although in the very strict sense the greater rate of the synthesis is sufficient justification of their stimulative effect in these directions.

The physiological rôle of these elements thus appears to be of no definite nature inasmuch as the stimulative and beneficial effect in one direction is counteracted by the baneful effect in others and as such it becomes a difficult task to stress with any degree of certainty the exact physiological rôle of these elements.

Summary

The investigation reported in the present paper is taken up with a view to studying the effect of some of the so-called non-essential elements employed in the form of their respective chlorides. *Triticum vulgare* (*var.* Pusa 4) has been used for experimentation. The different cultures have been divided into a number of series, the first being designated as the control, while the rest include the chlorides supplied in four different concentrations ranging from 0·01 to 0·0001*M* strength. The experiments have been conducted under rigidly controlled environmental conditions. Factors other than the concentration of chlorides are kept above their limiting value such that the variations observed may wholly be accounted for on the basis of the concentration of the various chlorides used for experimental purpose. The changes introduced as a result of the application of the various chlorides have been studied in terms of certain morphological and compositional variations, *viz.*, shoot and root length, assimilating surface, dry matter production, and the different carbohydrates and nitrogen accumulation, etc. The following inferences are drawn from the observations recorded herein :—

Salts	Concentrations	Effect
$CoCl_2$	0·01*M*	Extreme toxicity ; a characteristic coloration of leaves ; plants die within thirty-six hours of application.
	0·005*M*	Severe toxicity ; plant survive for about a week after application.
	0·0005*M*	Mild toxic effect ; plants survive for about three weeks after application.
	0·0001*M*	Feeble toxicity ; plants complete their life cycle with earlier ear formation but final dry matter yield is below the control.

Salts	Concentrations	Effect
ICl	0·01*M*	Severe toxicity ; plants die within a week after addition.
	0·005*M*	Severe toxicity leading to the death of plants two weeks after application.
	0·0005*M*	Lessened toxicity ; plants survive for about three weeks after application.
	0·0001*M*	Mild toxicity ; plants complete their life-cycle but the final dry matter yield is below the control.
$ZnCl_2$	0·01*M*	Severe toxicity leading to the death of plants two weeks after application.
	0·005*M*	Lessened toxicity ; plants survive for about three weeks after application.
	0·0005*M*	A remarkable stimulative effect ; plants survive for their whole life-cycle ; plant growth and final dry matter yield is far above he normal.
	0·0001*M*	Stimulative effect resulting in the vigorous growth of plants and increased final dry matter yield.
$AlCl_3$	0·01*M*	Toxic effect ; plants die four weeks after application.
	0·005*M*	Toxic effect ; most of the plants die without completing their life-cycle while the remaining ones exhibit growth and final dry matter yield below the control.
	0·0005*M*	Stimulative effect ; plants survive for their whole life-cycle but with a final dry matter yield which is below the control.
	0·0001*M*	A better stimulative effect ; the growth and final dry matter yield is far above the normal.
$MnCl_2$	0·01*M*	Toxic effect ; plants die before completing their life-cycle. Poor stunted growth with greatly diminished dry matter yield.
	0·005*M*	Toxic effect ; growth and final dry matter yield much below normal.
	0·0005*M*	Slight toxicity ; growth and final dry matter yield more or less equal to the normal.
	0·0001*M*	Stimulative effect ; plant growth and final dry matter yield are above the normal.
LiCl	0·01*M*	Toxic effect ; plants exhibit poor growth and final dry matter yield is below the normal.
	0·005*M*	No toxicity ; growth and final dry matter yield is more or less equal to the control.
	0·0005*M*	No toxicity ; the general vigour and final dry matter yield is almost equal to the normal,
	0·0001*M*	Retardation in growth and final dry matter yield which is below the control.
NaCl	0·01*M*	Stimulative effect. Better plant growth and greater final dry matter yield than the control.
	0·005*M*	Lessened stimulative effect. The growth and final dry matter yield is above the control.
	0·0005*M*	No stimulative influence. Final dry matter yield is more or less equal to the control.
	0·0001*M*	Slight retardation both in growth and final dry matter yield.

The accumulation of the different carbohydrates towards the close of the life-cycle varies with the type and concentration of the chloride supplied. The glucose content in sodium chloride under 0·01M is greater while in lithium, aluminium and manganese chlorides under 0·0005, 0·0005 and 0·0001, and 0·0001M concentrations respectively, it is almost equal to that of the control. The sucrose content is in almost all cases below the control. The starch content, in majority of the sets, is higher than that in the untreated plants. The total carbohydrates in case of sodium, lithium, zinc and manganese chlorides under 0·01, 0·005, 0·0005 and 0·0001M respectively, is higher than that in the control.

Nitrogen content in practically all the treated plants is lower than that in the control, zinc chloride being the only exception in which case 0·01 and 0·005M concentrations appear to be useful.

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TABLE I

Length of root and shoot, leaf area and dry weight per plant at successive stages of growth of Triticum vulgare (var. *Pusa 4*) *in different culture solutions*

Days	Root length (cm.)	Shoot length (cm.)	Leaf area (sq. cm.)	Dry weight (grm.)
Lithium chloride (0·01*M*)				
7	7·8	6.3	8·5	0·0202±0·0004
14	8·2	7·5	14.2	0·0432±0·001
21	9·4	8·15	19.8	0·0861±0·001
28	11·7	11·45	24.65	0·1683±0·005
35	13·5	13·8	28·55	0.2871±0·003
42	13·7	14·5	30·7	0·3785±0·002
49	14·8	14·6	30·7	0·4462±0·011
56	14·8	14·6	30·7	0·4802±0·015
Lithium chloride (0·005 *M*)				
7	7·9	6·1	8·8	0·0202±0·0004
14	8·3	7·2	15·6	0·0687±0·001
21	9·1	8·8	22·4	0·1087±0·001
28	12·2	14·4	27·2	0·2052±0·005
35	13·9	18·3	30·68	0·3201±0·006
42	14·5	20·5	32·54	0·4280±0·011
49	17·2	22·6	33·4	0·4882±0·014
56	17·2	22·6	33·4	0·5430±0·015
Lithium chloride (0·0005 *M*)				
7	7·7	6·3	8·7	0·0202±0·0004
14	8·1	8·1	16·2	0·0684±0·001
21	9·7	13·5	22·9	0·1287±0·002
28	12·8	19·3	29·5	0·2321±0·004
35	13·7	22·9	35·8	0·3387±0·006
42	14·9	23·8	40·20	0·3868±0·008
49	18·7	24·9	42·0	0·5361±0·015
56	18·7	24·9	42·0	0·5470±0·015
Lithium chloride (0·0001 *M*)				
7	8·1	6·0	8·6	0·0202±0·0004
14	8·4	7·1	14·4	0·0587±0·001
21	9·1	11·5	21·6	0·0927±0·002
28	11·9	16·9	28·2	0·1866±0·004
35	13·2	19·5	32·6	0·3057±0·007
42	14·6	20·2	38·8	0·4108±0·011
49	17·5	21·8	40·0	0·4603±0·012
56	17·5	21·8	40·0	0·4960±0·012

TABLE I—*contd.*

Days	Root length (cm.)	Shoot length (cm.)	Leaf area (sq. cm.)	Dry weight (grm.)
Sodium chloride (0·01 *M*)				
7	8·0	6·2	9·6	0·0202±0·0004
14	8·4	8·0	18·9	0·0685±0·001
21	9·8	13·7	26·7	0·1601±0·004
28	11·9	22·4	33·4	0·2785±0·006
35	13·2	24·8	38·6	0·3792±0·009
42	14·6	25·6	39·8	0·4801±0·012
49	16·8	26·8	44·5	0·5602±0·015
56	17·5	26·8	44·5	0·6034±0·016
Sodium chloride (0·005 *M*)				
7	8·3	6·4	9·4	0·0202±0·0004
14	8·6	7·9	18·1	0·0621±0·001
21	9·4	12·8	24·3	0·1502±0·004
28	15·1	20·9	29·9	0·2504±0·006
35	16·8	23·1	35·4	0·3400±0·011
42	17·3	23·8	36·9	0·4250±0·012
49	20·8	25·2	42·7	0·5288±0·013
56	20·8	25·2	42·7	0·5860±0·014
Sodium chloride (0·0005 *M*)				
7	8·1	6·1	9·7	0·0202±0·0004
14	8·5	7·2	18·0	0·0587±0·001
21	9·2	11·9	23·8	0·1483±0·003
28	15·7	18·6	29·2	0·2396±0·006
35	16·9	22·8	34·7	0·3107±0·008
42	18·7	23·4	35·8	0·4003±0·011
49	21·5	24·9	42·0	0·5018±0·013
56	21·5	24·9	42·0	0·5420±0·014
Sodium chloride (0·0001 *M*)				
7	7·8	5·9	9·4	0·0202±0·0004
14	8·3	7·0	17·9	0·0584±0·001
21	9·5	12·1	23·8	0·1407±0·004
28	16·1	18·4	28·8	0·2374±0·006
35	17·2	22·3	34·2	0·3002±0·007
42	18·7	23·1	35·4	0·3946±0·011
49	21·2	24·1	41·2	0·4973±0·012
56	21·2	24·1	41·2	0·5410±0·014
Aluminium chloride (0·01 *M*)				
7	7·7	6·1	8·6	0·0202±0·0004
14	7·7	6·6	16·5	0·0405±0·001
21	7·8	6·6	18·7	0·0812±0·002
28	7·8	6·7	18·7	0·1457±0·004
35	7·8	6·7	18·7	0·2368±0·006

(Plants were killed due to toxicity of this solution).

TABLE I—*contd.*

Days	Root length (cm.)	Shoot length (cm.)	Leaf area (sq. cm.)	Dry weight (grm.)
Aluminium chloride (0·005 M)				
7	8·0	6·2	8·9	0·0202±0·0004
14	8·0	6·9	16·3	0·0501±0·001
21	8·1	9·2	20·1	0·0924±0·002
28	8·2	13·7	22·4	0·1532±0·004
35	8·2	15·9	22·9	0·2503±0·006
42	8·2	16·4	23·6	0·3321±0·008
49	8·3	16·8	23·8	0·4001±0·011
56	8·3	16·8	24·8	0·4120±0·011
Aluminium chloride (0·0005 M)				
7	8·2	6·2	8·5	0·0202±0·0004
14	8·2	7·2	18·9	0·0681±0·001
21	8·3	9·4	25·8	0·1201±0·003
28	8·3	15·3	35·1	0·2004±0·005
35	8·4	19·1	39·2	0·3071±0·007
42	8·4	20·8	41·3	0·4172±0·011
49	8·5	22·2	42·2	0·4853±0·012
56	8·5	22·2	42·2	0·5102±0·013
Aluminium chloride (0·0001 M)				
7	7·9	6·4	8·8	0·0202±0·0004
14	7·9	8·0	20·5	0·0675±0·001
21	8·0	14·8	26·2	0·1052±0·002
28	8·3	16·2	36·2	0·1957±0·005
35	8·4	19·9	40·4	0·3201±0·008
42	8·6	21·8	42·7	0·4507±0·012
49	8·7	24·0	44·0	0·5510±0·014
56	8·7	24·0	44·0	0·5910±0·014
Manganese chloride (0·01M)				
7	7·7	5·8	9·7	0·0202±0·0004
14	9·2	7·1	15·8	0·0403±0·001
21	11·4	7·2	18·2	0·0687±0·001
28	15·1	7·3	19·4	0·1403±0·004
35	15·9	7·4	20·2	0·2709±0·006
42	16·2	7·5	20·2	0·3007±0·007
49	18·2	7·5	20·2	0·4100±0·011
(Plants were killed due to toxicity of this solution).				
Manganese chloride (0·005M)				
7	7·9	5·9	9·3	0·0202±0·0004
14	8·7	7·2	15·9	0·0428±0·001
21	10·8	8·4	20·3	0·0803±0·002
28	13·9	10·7	21·8	0·1521±0·004
35	15·2	10·8	22·2	0·2403±0·006
42	15·7	10·9	22·5	0·3285±0·008
49	18·2	10·9	22·5	0·4250±0·011
56	18·3	10·9	22·5	0·4480±0·012

TABLE I—*contd.*

Days	Root length (cm.)	Shoot length (cm.)	Leaf area (sq. cm.)	Dry weight (grm.)
Manganese chloride (0·0005M)				
7	7·8	6·2	9·2	0·0202±0·0004
14	8·6	7·3	17·4	0·0489±0·001
21	13·8	9·1	26·4	0·0952±0·002
28	15·6	14·5	33·6	0·1802±0·004
35	19·3	18·9	38·9	0·2681±0·006
42	19·5	21·8	40·6	0·3783±0·009
49	21·4	24·2	42·0	0·4857±0·012
56	21·4	24·2	42·0	0·5390±0·013
Manganese chloride (0·0001M)				
7	8·0	6·0	9·4	0·0202±0·0004
14	9·8	7·9	18·2	0·0598±0·001
21	14·5	13·7	28·3	0·1203±0·003
28	19·4	18·9	35·2	0·2210±0·005
35	20·8	22·7	40·4	0·3146±0·007
42	21·2	24·1	43·4	0·4638±0·012
49	24·6	25·8	43·9	0·5287±0·013
56	24·8	25·8	44·0	0·5840±0·014
Zinc chloride (0·01M)				
7	7·8	6·0	8·8	0·0202±0·0004
14	7·9	6·4	11·3	0·0652±0·001
21	8·3	6·9	12·1	0·1482±0·004
(Plants were killed due to toxicity of this solution)				
Zinc chloride (0·005M)				
7	8·2	6·2	9·1	0·0202±0·0004
14	8·6	6·3	13·2	0·0601±0·001
21	9·1	7·0	15·7	0·1268±0·003
28	9·5	7·4	16·4	0·2160±0·005
(Plants were killed due to toxicity of this solution)				
Zinc chloride (0·0005M)				
7	8·0	6·9	9·4	0·0202±0·0004
14	8·6	7·8	18·8	0·0599±0·001
21	9·8	12·9	27·8	0·1187±0·003
28	11·2	20·7	34·6	0·1943±0·004
35	12·5	23·3	49·8	0·3361±0·008
42	13·1	23·7	43·8	0·4604±0·012
49	15·7	24·7	44·5	0·5764±0·014
56	15·8	24·7	44·5	0·6130±0·015

TABLE I—*contd.*

Days	Root length (cm.)	Shoot length (cm.)	Leaf area (sq. cm.)	Dry weight (grm.)
Zinc chloride (0·0001M)				
7	7·9	6·1	8·7	0·0202±0·0004
14	8·3	7·9	17·5	0·0578±0·001
21	9·9	13·7	25·6	0·1125±0·003
28	12·4	15·9	32·8	0·1843±0·004
35	13·8	21·1	38·6	0·3201±0·007
42	14·6	22·2	42·0	0·4423±0·011
49	17·8	23·1	42·8	0·5280±0·013
56	17·8	23·2	42·8	0·5820±0·014
Cobalt chloride (0·01M)				
(Plants were killed within 36 hours in this solution)				
Cobalt chloride (0·005 M)				
7	7·9	6·2	8·7	0·0202±0·0004
14	7·9	6·4	9·5	0·0361±0·0007
(Plants were killed due to the toxicity of this solution).				
Cobalt chloride (0·0005M)				
7	8·4	6·4	8·8	0·0202±0·0004
14	8·6	6·6	13·5	0·0501±0·001
21	8·7	6·6	20·2	0·0987±0·002
28	8·7	6·9	23·8	0·1262±0·003
(Plants were killed due to toxicity of this solution)				
Cobalt chloride (0·0001M)				
7	8·2	6·1	9·2	0·0202±0·0004
14	8·2	7·5	17·8	0·0632±0·001
21	8·3	13·2	25·7	0·1183±0·003
28	8·3	16·7	33·8	0·1703±0·004
35	8·4	18·9	37·4	0·2681±0·006
42	8·5	18·9	38·9	0·3786±0·009
49	8·6	20·2	39·2	0·4552±0·012
56	8·6	20·5	39·4	0·5120±0·013
Iodine chloride (0·01M)				
7	8·2	5·9	8·6	0·0202±0·0004
14	8·2	6·0	10·0	0·0387±0·0007
(Plants were killed due to toxicity of this solution).				
Iodine chloride (0·005M)				
7	8·1	6·1	8·9	0·0202±0·0004
14	8·2	6·3	10·7	0·0485±0·0009
21	8·3	6·4	11·2	0·0691±0·001
(Plants were killed due to toxicity of this solution).				

TABLE I—*concld.*

Days	Root length (cm.)	Shoot length (cm.)	Leaf area (sq. cm.)	Dry weight (grm.)
Iodine chloride (0·005M)				
7	8·4	6·4	8·8	0·0202±0·0004
14	8·5	6·9	13·0	0·0512±0·001
21	8·6	7·4	20·1	0·0843±0·002
28	8·6	8·1	23·5	0·1281±0·003
(Plants were killed due to toxicity of this solution)				
Iodine chloride (0·0001M)				
7	8·3	6·2	9·0	0·0202±0·0004
14	8·3	8·6	18·0	0·0607±0·001
21	8·4	9·8	25·9	0·1102±0·003
28	8·4	17·9	34·2	0·1581±0·004
35	8·5	20·7	38·0	0·2680±0·006
42	8·5	21·5	39·8	0·3810±0·009
49	8·6	22·2	40·1	0·4205±0·011
56	8·7	22·2	40·5	0·4521±0·012
Control				
7	8·2	6·4	9·7	0·0202±0·0004
14	8·7	7·7	18·8	0·0601±0·001
21	9·9	12·9	26·2	0·1085±0·003
8	16·8	19·1	32·8	0·1821±0·004
35	19·5	20·8	37·2	0·2532±0·006
42	20·2	22·5	38·7	0·3487±0·008
49	22·6	24·4	42·5	0·4501±0·012
56	22·6	24·4	42·8	0·5480±0·013

TABLE II

Content of carbohydrate fractions, and total nitrogen at the final stage of growth of Triticum vulgare (var. *Pusa 4*) *in different cultural solutions*

Treatment	Percentage (dry-weight basis)					
	Glucose	Sucrose	Starch	Total carbo-hydrate	Total nitrogen	Remarks*
LiCl (0·01M) . .	14·32	6·84	12.60	33·76	3·780	56
,, (0·005M) . .	15·97	7·23	15·14	38·34	3·720	56
,, (0·0005M) .	15·82	9·85	16·43	42·10	3·590	56
,, (0·0001M) .	13·94	7·64	14·20	35·78	3·680	56

* The number of this column indicates the number of days the plants grew in the different culture solutions.

TABLE II—*contd.*

Treatment	Percentage (dry-weight basis)					
	Glucose	Sucrose	Starch	Total carbo-hydrate	Total nitrogen	Remarks*
NaCl (0·01*M*)	17·43	10·96	15·625	44·015	3·306	56
,, (0·005*M*)	16·15	8·84	15·42	40·41	3·306	56
,, (0·0005*M*)	15·63	9·62	13·21	38·46	3·421	56
,, (0·0001*M*)	14·42	8·57	11·88	34·87	3·389	56
$AlCl_3$ (0·01*M*)	15·35	5·27	7·93	28·55	3·502	35
,, (0·005*M*)	15·86	8·20	11·03	35·09	3·750	56
,, (0·0005*M*)	16·12	9·87	13·87	39·86	3·680	56
,, (0·0001*M*)	16·48	10·62	13·65	40·85	3·890	56
$MnCl_2$ (0·01*M*)	12·29	6·48	14·12	33·25	3·783	49
,, (0·005*M*)	13·85	7·93	15·42	37·20	3·738	56
,, (0·0005*M*)	14·89	10·21	13·49	38·59	3·649	56
,, (0·0001*M*)	16·57	10·94	14·24	41·75	3·812	56
$ZnCl_2$ (0·01*M*)	12·29	6·82	14·20	33·31	4·587	21
,, (0·005*M*)	31·85	5·79	10·00	29·64	4·672	28
,, (0·0005*M*)	14·88	11·76	14·84	41·48	3·685	56
,, (0·0001*M*)	14·20	10·12	13·89	38·21	3·703	56
$CoCl_2$ (0·01*M*)	8·21	9·19	7·00	24·40	3·515	2
,, (0·005*M*)	11·38	8·01	12·50	31·89	3·094	14
,, (0·0005*M*)	13·57	7·15	10·87	31·59	3·421	28
,, (0·0001*M*)	14·92	11·94	13·48	40·34	3·613	56
ICl (0·01*M*)	8·63	8·12	11·02	27·77	3·248	14
,, (0·005*M*)	10·41	7·02	16·50	33·93	2·956	21
,, (0·0005*M*)	14·12	6·60	9·31	30·03	2·876	28
,, (0·0001*M*)	15·05	6·95	9·02	31·02	3·051	56
Control	16·58	11·16	13·50	41·24	3·813	56

* The number of this column indicates the number of days the plants grew in the different culture solutions.

A BIOCHEMICAL STUDY OF THE PADDY SOILS OF BENGAL WITH SPECIAL REFERENCE TO THEIR NITROGEN-FIXING CAPACITIES

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(Received for publication on 3rd January 1936)

Fertilisation of rice fields is seldom practised in Bengal ; yet the crop grows here consistently year after year without showing any change in the fertility of the soil. Obviously, therefore, there is the natural recuperation of soil nitrogen. Howard [1924] suggests that the abundant growth of algal film very often observed on the surface of the water-logged paddy fields, working conjointly with the bacteria within it, may fix certain amount of nitrogen. Experiments in this laboratory [De, 1934] have shown that when water-logged soil is exposed to sunlight, a considerable amount of nitrogen is fixed. This, however, will form the subject of a future communication. The present investigation is undertaken to study the fixation of nitrogen in the paddy soils under dry cultivated conditions.

EXPERIMENTAL

Six soil samples, representative of paddy soils in Bengal, were selected for the present work. They were obtained from the unmanured paddy fields of the following districts—Barisal, Birbhum, Dacca, Faridpur, Rajshahi and Rangpur. Soils from Dacca and Faridpur were locally collected while the remaining four were kindly supplied by the respective District Agricultural Officers.

Samples (0 in.—6 in.) were air-dried, passed through one-mm. sieve and then preserved in stoppered glass containers for use in the following experiments. A short description of the type of the soil, the pH and the C : N ratio are given in Table I.

TABLE I

Soil	Type	C : N	pH
Barisal	Deltaic . . .	8·8	6·66
Birbhum	Lateritic . . .	10·3	7·26
Dacca	Lateritic . . .	9·3	5·11
Faridpur	Alluvial . . .	10·3	8·19
Rajshahi	Calcareous . . .	8·8	8·35
Rangpur	,,	10·2	6·36

NITROGEN-FIXING CAPACITIES OF THE SOILS

In the present experiment the nitrogen-fixing capacities of the above six soils were determined by the use of both large silica gel plate method of Winogradsky [1926] and the solution method. In the latter method the cultures are generally incubated for a long period. Gainey and Briscoe [1933] have shown that the differences in the relative nitrogen-fixing capacities of soils, as determined by the amount of nitrogen fixed in culture media, can be detected only when the incubation period is relatively short ; on prolonged incubation these differences disappear. It is therefore, necessary first of all to select a period of incubation suitable for determining the nitrogen-fixing capacity of soils by the solution method.

Hundred-c.c. lots of Ashby's medium, after sterilisation, were inoculated with one-gram portions of Barisal, Faridpur and Rajshahi soils which are known to contain *Azotobacter*. The cultures were incubated at 33°C. and at the end of 4th, 5th and 7th day, the amount of nitrogen fixed were determined. The results are given in Table II.

TABLE II

Showing the effect of varying lengths of incubation period on the fixation of nitrogen in the culture solution

Soil	Period of incubation		
	4 days	5 days	7 days
Barisal	5·6	6·4	6·9
Faridpur	6·2	7·5	8·6
Rajshahi	7·6	8·2	8·7

The figures represent milligrams of nitrogen fixed and are the averages of three determinations.

It will be seen that although marked differences in the nitrogen-fixing capacities of the three soils were observed after an incubation period of four days, these differences gradually faded as the incubation was prolonged with the result that after seven days the differences between Faridpur and Rajshahi soils entirely disappeared. It was, therefore, decided to use an incubation period of four days in all studies on the fixation of nitrogen with solution method.

The nitrogen-fixing capacities of the above soils are given in Table III.

TABLE III

Showing the nitrogen-fixing capacities of the soils when tested by (1) silica gel plate method, (2) solution method

Soil	Mg. of N fixed per plate	Mg. of N fixed in 100 c.c. of solution
Faridpur	9·9	6·2
Rajshahi	8·5	7·7
Barisal	5·3	5·6
Birbhum	1·3	Very small
Rangpur	1·3	,, ,,
Dacca	1·2	,, ,,

FIXATION OF NITROGEN IN SOIL

The results recorded in Table III while indicating the relative activities of the nitrogen-fixing flora of different soils, give absolutely no idea as to the amount of nitrogen that may be fixed in the soil itself under natural conditions. Apart from the presence or absence of active nitrogen-fixing organisms, the fixation of nitrogen in soil is controlled by other factors such as the supply of available phosphate or nitrogen, reaction of the soil and the competition amongst the organisms for available energy. The object of the following experiment was to find out whether measurable amounts of nitrogen are fixed in the soils when they are kept under optimum conditions of temperature and moisture. It will be noted that these conditions correspond to those prevailing in the paddy fields during the winter months when the soils remain dry and fallow. A study of the effect of the addition of plant residues on the fixation of nitrogen in paddy soils was considered important as the roots, stubbles and a portion of the straw remaining in the fields after harvest, might stimulate the growth of the nitrogen-fixing organisms, being used up as energy materials. The effect of the addition of lime and phosphate on the fixation of nitrogen was also studied. The details of the experiment are as follows :

Three 120-grm. portions of each of the air-dried soils were distributed in three beakers of 400 c.c. capacity which then received the following treatments. :—

(1) No treatment (120 grms. of soil).

(2) As (1) + 0·1 grm. K_2HPO_4 + 0·1 grm. $CaCO_3$.

(3) As (2) + 2 grms. of powered rice straw.

After the contents of each beaker were thoroughly mixed, the moisture was adjusted to eighteen per cent on air-dry basis. The beakers were then put in the incubator at a temperature of 33°C. Every alternate day the loss of moisture was made up and the soil thoroughly stirred to facilitate aeration. Total nitrogen was determined at intervals of twenty, forty and sixty days. The results are given in Table IV.

TABLE IV

Showing the amounts of nitrogen fixed in different soils

(Milligrams of nitrogen in 10 grms. of dry soil)

Soil	At start			After 20 days			After 40 days			After 60 days			Mg. of N fixed in 60 days		
	0	Ca+P	Ca+P+S	0	Ca+P	Ca+P+S	0	Ca+P	Ca+P+S	0	Ca+P	Ca+P+S	0	Ca+P	Ca+P+S
Faridpur . .	6·4	6·4	7·2	6·9	6·9	7·7	6·9	6·9	7·7	6·9	6·9	7·7	0·5	0·5	0·5
Dacca . .	8·3	8·3	9·0	8·3	8·6	9·1	8·6	8·6	9·4	8·9	8·9	9·6	0·6	0·6	0·6
Barisal . .	13·1	13·1	13·9	13·1	13·1	13·8	13·1	13·2	14·0	13·3	13·4	14·0	0·2	0·3	0·1
Rangpur . .	9·3	9·3	10·1	9·0	9·0	9·9	9·3	9·3	10·4	9·5	9·6	10·3	0·2	0·3	0·2
Rajshahi . .	10·3	10·3	11·1	10·3	10·4	11·2	10·4	10·4	11·3	10·3	10·4	11·3	0·0	0·1	0·2
Birbhum . .	5·6	5·6	6·4	5·6	5·5	6·5	5·6	5·6	6·5	5·6	5·5	6·5	0·0	—0·1	0·1

O = No treatment. Ca + P = Treated with phosphate and lime. Ca + P + S = Treated with phosphate, lime and straw.

TABLE V

From these results the amounts of nitrogen fixed in pounds per acre are calculated, assuming that the weight of soil per acre up to a depth of 6 in. is 2,000,000 lbs.

Amounts of nitrogen fixed in different soils expressed as lbs. per acre

Soil	Control	Ca + P	Ca + P +S
Dacca	120	120	120
Faridpur	100	100	100
Barisal	40	60	20
Rangpur	40	60	40
Rajshahi	0	10	40
Birbhum	0	—10	10

In the interpretation of the above results, difference more than 0·14 mg. of nitrogen in ten grms. of soil or twenty-eight lbs. in an acre was considered significant.

From the foregoing observations the following facts emerge :—

(1) The amounts of nitrogen fixed in the first four soils are more than the average amount of nitrogen removed annually by the crop. (28 lbs. per acre).

(2) The addition of lime and phosphate in the soil had no marked effect on the fixation of nitrogen. This shows that the lack of available phosphate or the unfavourable soil reaction are not the factors inhibiting the fixation of nitrogen in these soils.

(3) Excepting in Rajshahi and Barisal soils, the addition of straw had no effect on nitrogen fixation. In the former soil, it has stimulated nitrogen fixation but in the latter its effect was depressive.

The results in Tables III and V show that the nitrogen-fixing capacity of a soil, as determined by the silica gel plate method or solution method, may give an entirely wrong idea about the actual fixation of nitrogen in the soil itself under natural conditions. For example, Rajshahi soil fixed the largest amount of nitrogen in culture solution and Dacca soil the least but their positions were reversed in Table V. The addition of straw did not bring about an increased fixation of nitrogen in most of the soils, indicating that the energy material already present was sufficient. Some soils such as Rajshahi and Birbhum, in spite of the conditions being made favourable for nitrogen fixation by the addition of phosphate, lime and straw, did not show much improvement in the fixation of nitrogen. It would thus appear that certain soils, even when harbouring beneficial flora, *e.g.*, *Azotobacter*, may become permanently inactive in fixing nitrogen and that treatment of such soils is not likely to improve their nitrogen-fixing power. Factors responsible for such infertility of the soil towards the nitrogen-fixing flora are, however, unknown at present. The fixation of large amount of nitrogen in Dacca soil which does not contain *Azotobacter* indicates the possibility of existence in paddy soils of very active nitrogen-fixing organisms which have not yet been discovered.

INFLUENCE OF SUNLIGHT ON THE FIXATION OF NITROGEN IN SOIL

In the previous experiment on the fixation of nitrogen in the paddy soils, one important factor has been ignored,namely the influence of sunlight on the fixation of nitrogen in soil. A study of this factor is important inasmuch as some workers have claimed that the blue green algae of soil are capable of fixing atmospheric nitrogen either independently or in symbiosis with nitrogen-fixing organisms. As the influence of algae on the fixation of nitrogen was not manifest in the above experiment due to the incubation of the soil in the dark (incubator), it was proposed to conduct another experiment to see if, on incubating the soil in the light and thus facilitating the growth of algae, the fixation of nitrogen in the soil could be increased. For this purpose 100-grm. portions of Faridpur soil were taken in a number of beakers with covers some of which were thickly painted outside by Japan-black. The moisture content of the soil was maintained at eighteen per cent throughout the experiment. The results of nitrogen determinations showed that there is no significant difference in fixation of nitrogen in the two treatments after exposure to sunlight for a month.

INFLUENCE OF WATER-LOGGING ON THE NITROGEN-FIXING CAPACITY OF SOIL

As rice fields remain submerged for a long period during the growth of the crop, it was thought that water-logging might profoundly modify their nitrogen-fixing capacities by altogether destroying the strictly aerobic nitrogen-fixing organism and by encouraging the growth of those thriving well under anaerobic conditions. To investigate this point, the following experiment was done in which the nitrogen-fixing capacity of dry cultivated soil was compared with that of the corresponding water-logged soil by the solution method using an incubation period of four days.

One hundred grm. of Faridpur soil were placed in a beaker and incubated at 33°C. after the moisture content had been adjusted to eighteen per cent on dry basis. The soil was frequently stirred to facilitate gas exchanges and the loss in moisture was made up by the periodic additions of water. At different intervals, samples were withdrawn by a sterile cork borer and after having determined the moisture contents, one-gram portions of soil on dry basis were inoculated into each of three flasks containing 100 c.c. of Ashby's mannite solution. After four days' incubation at 33°C., the contents of the flasks were analysed for total nitrogen. For the determination of the nitrogen-fixing capacity of the water-logged soil, a number of test-tubes each containing one gram of soil on dry basis and 5 c.c. of distilled water were incubated at 33°C. For each experiment, three test-tubes were removed and the contents, after thorough shaking, were separately poured into three flasks each containing 100 c.c. of mannite solution. Total nitrogen was determined as before after four days' incubation. The results show that the activities of the nitrogen-fixing organisms of soil were depressed to some extent under water-logged conditions.

TABLE VI

Showing the effect of water-logging on the nitrogen-fixing capacity of soil. Figures represent milligrams of nitrogen fixed in 100 c.c. of culture solution.

Days after incubation	Dry cultivated soil	Water-logged soil	Difference
3	5·8	5·0	0·8
10	7·6	5·8	1·8
15	8·0	6·0	2·0
20	8·5	6·3	2·2

Figures are the averages of three flasks.

BACTERIOLOGICAL EXAMINATION OF THE SOILS

The fixation of nitrogen in ordinary arable soils is supposed to be the result of the activities of *Azotobacter* and *B. amylobacter* which commonly occur in soils and are considered to be very active nitrogen fixers. There is another group of nitrogen-fixing organisms known as *B. radiobacter* which is present in soil in so great abundance that its role in the fixation of nitrogen in soil cannot be overlooked. But in the paddy soils, because of the swamp conditions prevailing for about half the year, the fixation of nitrogen might not be brought about by the same agents as those in normal soils. Accordingly the determination of the relative abundance of the above three groups of organisms in different soils was made to find out whether the amounts of nitrogen fixed were related to the number of nitrogen-fixing organisms in those soils. The following methods were used for counting the organisms :—

Azotobacter.—The method is essentially the same as that described by Winogradsky [1926], the only difference being that agar plates were used in place of silica gel plates. It has been shown by Curie [1932] that result obtained with agar plates were sufficiently uniform to permit a quantitative comparison of the *Azotobacter* flora of different soils.

B. radiobacter.—The method originally devised by Smith [1928] and subsequently modified by Starky [1929] was used for counting the number of *radiobacter*. In the original method of Smith, the diluted soil suspension (100 c.c.) is treated with 0·5 c.c. of 0·1 per cent crystal violet and one-c.c. portions of the suspension are plated out. Starky's modification consists in adding the dye (one c. c. of 0·1 per cent solution) to 100-c. c. portions of the medium immediately before plating. It may be noted that the concentration of dye in Starky's medium is ten times greater than in Smith's. The advantage of Starky's method is that the colonies appearing on the plates are mostly of *B. radiobacter*, which can be easily distinguished from the contaminating growth.

B. amylobacter.—The number of *B. amylobacter* in soil was determined by dilution method adapted from one in use in the General Microbiological Department of the Rothamsted Experimental Station. The method depends on setting up duplicate cultures containing one c.c. of each of a series of thirteen half-and-half dilutions of a well-shaken soil suspension of known strength in 15 c.c. of a nitrogen-free agar medium [No 11, Waksman and Fred, 1928]. After incubating anaerobically at 33°C. for a week, the cultures were examined. By noting the number of cultures from which the organism is absent it is possible to estimate statistically the number of orgnisms per gram of soil. The statistical tables constructed by Fisher [1922] were used for this purpose. The following table gives a detailed record of the cultures made from Faridpur soil in order to illustrate the method of setting up the cultures for the purpose of obtaining information about the frequency of the organism in soil.

TABLE VII

Degree of dilution of soil suspension	Culture number	Growth
1 : 50	1	+
	2	+
1 : 100	1	+
	2	+
1 : 200	1	+
	2	+
1 : 400	1	+
	2	+
1 : 800	1	+
	2	+
1 : 1600	1	+
	2	+
1 : 3200	1	+
	2	+
1 : 6400	1	+
	2	+
1 : 12800	1	—
	2	—
1 : 25600	1	+
	2	—
1 : 51200	1	—
	2	—
1 : 102400	1	+
	2	—
1 : 204800	1	—
	2	—

Number of sterile tubes=8.

Number of bacteria per gram of soil=10,600.

(From Fisher's table).

TABLE VIII

Showing the number of nitrogen-fixing organisms per gram of soil

Soil	Azotobacter colonies	*B. amylobacter*	*B. radiobacter*
Faridpur	1310	10,600	48,125
Rajshahi	2740	23,000	34,375
Barisal	144	118,000	5,000
Birbhum	Nil	2,600	25,000
Rangpur	,,	22,000	Very few
Dacca	,,	32,000	Nil

SEARCH FOR NEW NITROGEN-FIXING ORGANISMS

It has already been pointed out that the organisms fixing nitrogen in the paddy soils might not be the same as those occurring normally in ordinary dry cultivated soils. Justification for such an assumption is further obtained from the fact that Dacca soil fixed the greatest amount of nitrogen (Table V) in spite of its low content of nitrogen-fixing flora as compared with those in Faridpur, Barisal and Rajshahi soil (Table VIII). Accordingly a search was made for the presence of new nitrogen-fixing organisms in all the six soils used in the present investigation. For isolation of the organisms both Winogradsky's nutrient silica gel plates and Ashby's agar plates were inoculated with small particles of soil. The plates were then incubated at 33°C. and examined daily. After six days' incubation it was noticed that a large number of raised and transparent colonies were appearing along with those of *Azotobacter* in the plates inoculated with Faridpur soil. The colonies were so tenacious that a part of it could not be removed by rubbing with platinum needle. Microscopic examination of the growth showed the presence of very large, encapsulated, spore-forming rods. For purification, a little growth was boiled with sterile water for two minutes and the hot suspension plated on Ashby's agar. In about three days, raised transparent colonies appeared all over the plates. The process was repeated once more to ensure the purity of the culture. The organism made very good growth on Ashby's agar plate but the growth in Ashby's solution was relatively much poor. The organism which has the following characteristics does not appear to have been described in Bergey's manual.

1. Large encapsulated rods generally occurring in packets; Length 5.6μ with the capsule and 1.5μ without capsule.
2. Gram-positive and non-motile.
3. Spore-forming.
4. Strictly aerobic.
5. Nitrate reduced to nitrite.

6. Formation of gas and acid in nitrogen-free glucose medium.
7. Milk curdled.
8. Broth slightly turbid.
9. Fair growth on potato, colonies greyish brown in colour.
10. Amount of nitrogen fixed on agar plate for every gram of mannite decomposed—5 mgs.

Dacca soil.—On both silica gel plates and Ashby's agar plates a large number of milk-white, raised and opaque colonies appeared after three week's incubation. A little growth was microscopically examined and was found to consist of spherical cells occurring in pairs—resembling in appearance the typical *Azotobacter* cells. The organism is non-motile and does not produce spore or pigment. It is stained only with difficulty by aniline dyes. In spite of various attempts the organism could not be obtained in pure culture. It seems to be a new species probably belonging to the genus *Azotobacter.*

Rajshahi and Barisal soils—The only important growth on the plate was that of *Azotobacter.*

Birbhum soil :—There was very little growth on silica gel plate.

SUMMARY

1. Fixation of nitrogen was studied with six paddy soils obtained from different districts of Bengal.

2. Amounts of nitrogen fixed in four of the soils were more than the amount removed annually by the crop.

3. The addition of phosphate or lime in the soil had no marked effect on nitrogen fixation.

4. On adding straw, nitrogen fixation was stimulated only in one of the soils.

5. Sunlight has no effect on the fixation of nitrogen in soil.

6. Water-logging was found to inhibit the activities of the nitrogen-fixing organisms of soils.

7. The number of nitrogen-fixing organisms in different soils were counted. They were present in large number in most of the soils.

8. A new nitrogen-fixing organism was isolated from Faridpur soil.

9. In determining the nitrogen-fixing capacity of soil by solution method, differences in the nitrogen-fixing abilities can be detected when the incubation period is relatively short (four days).

The authors' thanks are due to Professor J. C. Ghosh and Dr. A. T. Sen for their interest in the work. Thanks are also due to the Imperial Council of Agricultural Research for financial help towards the purchase of necessary apparatus and maintenance of the salary of one of them (P. K. D.).

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BISMUTHATE METHOD FOR THE ESTIMATION OF MANGANESE IN PLANT MATERIAL

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(Received for publication on 23rd October 1935)

Since the recognition of the important role played in the metabolism of the plant by elements which occur in relatively small amounts the so-called " non-essential elements "—the attention of the analytical chemist has been drawn to the elaboration of methods for their accurate determination. Since these elements, though present in very minute amounts, are regarded as necessary for the proper growth and development of the plant and as important as those which constitute the greater portion of it, it has been felt that the methods used for their estimation must be sensitive and accurate enough to yield reliable results under a wide range of conditions. Manganese is one of these elements. The importance of manganese in increasing the oxygen-carrying power of oxidases was first recognised by Bertrand [1897], McCool [1913] ; and Mclean and Gilbert [1925] explain chlorosis as the result of the absence or deficiency of manganese. McHargue [1922] regards manganese as being concerned in nitrogen assimilation and protein synthesis and also to function with iron in the synthesis of chlorophyll.

In view of the important role played by manganese in plant metabolism, it is essential to have a reliable method for its accurate determination, specially when it is present in small amounts. In the literature there are mentioned a large number of methods ; colorimetric, gravimetric and volumetric, which have been advocated by different workers and used from time to time for the estimation of this element. Colorimetric methods, although capable of giving fairly accurate results, suffer from the serious disadvantage that all workers are not equally sensitive to slight changes in the intensity of tints. Gravimetric methods are believed to yield accurate results. This accuracy, however, diminishes when the elements to be estimated are present in minute quantities. In such cases, even an otherwise slight error, due to the solubility of precipitates or its contamination with impurities is greatly magnified. The volumetric methods are considered to be free from such defects and in case a satisfactory method for any particular estimation is available, it is to be preferred.

Schneider [1897] was the first worker to propose a volumetric method for the estimation of manganese. It was based upon the use of lead peroxide as an oxidising agent. Ibbetson and Brearley [1901], with certain modifications report to have successfully used this method in the analyses of white metal alloys. It was however, not before 1904, that the method was recommended for general application by Blair, who studied it in detail. Davidson and Capen [1931] made a comparative study of this, and the persulphate and periodate colorimetric methods as well as the gravimetric method. They found that of all the above methods the sodium bismuthate method gave the lowest results, exceptionally high figures being obtained with the gravimetric method. For the estimation of manganese in soils the bismuthate method has been in use in these laboratories for a number of years and has always given satisfactory results.

The suitability of this method for the analysis of plant material, as compared with the gravimetric method of the A. O. A. C. [1925], has been studied under a wide range of well-controlled conditions and it has been found that with some modifications in the technique proposed by Blair [1904] for the analysis of iron and manganese ores, the method gives satisfactory results.

METHOD

Preparation of extract

Finely ground plant material to the extent of 200 grams is carefully ignited at a dull red heat as completely as possible. The ash obtained is digested with strong hydrochloric acid for eight hours on a sand-bath, at a temperature just sufficient to cause occasional ebullition. When digestion is complete, the residue in the conical flask is transferred to a porcelain dish and evaporated to dryness on a water-bath, thus rendering silica insoluble. The contents of the dish are now extracted with hydrochloric acid, filtered through a hardened filter paper, washed with distilled water till free from chlorides and the filtrate made up to one litre. We prefer this method of preparing the extract to the one in which plant material is ashed separately for the estimation of each element or group of elements, in as much as the errors due to individual variations of sampling, which will be reflected in the ratios of different elements determined are eliminated when a large amount of the sample is taken, ashed and extracted in one lot ; also it saves much time, which is otherwise taken in ashing and extracting individual samples separately. Further, the ash obtained can be stored over any length of period without undergoing any change in its composition whereas the plant material is subject to attacks by insects and fungus and thus liable to changes in composition.

Estimation of manganese

Aliquot portions from the stock solution prepared as above are transferred to porcelain dishes, a few drops of concentrated nitric acid added and the whole evaporated to dryness on the water-bath. The residue in the dishes is now cooled, and after the addition of 2·5 c.c. of concentrated sulphuric acid is again heated on sand-bath till copious fumes of SO_3 begin to be evolved. This removes all traces of hydrochloric acid. The dishes are now cooled and the contents transferred to a conical flask of 300 c.c. capacity, with the aid of 50 c.c. of approximately 4 *N* nitric acid.* In order to assist solution, the liquid in the flasks is heated to boiling, care being taken to avoid loss of acid as far as possible. The temperature of the liquid is now brought to within 15° to 20°C., which after the addition of 0·5 gram of sodium bismuthate (B. D. H., A. R. preparation) is kept aside for half an hour and occasionally shaken. After keeping it undisturbed for about five minutes, fifty c.c. more of three per cent nitric acid, also free from nitrous acid, are added ; taking care that the temperature during the operation does not exceed 30°C. The solutions are now filtered through a mat of specially prepared asbestos(*), spread over a perforated porcelain disc fitted in a glass funnel and the residue washed with fifty c.c. of the same three per cent nitric acid, using small quantites at a time. The filtration and washing does not take more than five minutes. The resulting permanganate is reduced by adding a known volume of approximately *N*/100 ferrous ammonium sulphate solution (containing 50 c.c. of concentrated sulphuric acid per litre of solution) and the excess of ferrous salt is immediately titrated with *N*/100 potassium permanganate. A blank determination using the same amount of sodium bismuthate and nitric acid solution is also carried out simultaneously. The difference between the amount of centinormal permanganate solution used for the two titrations gives the amount of manganese present ; one c.c. of *N*/100 $KMnO_4$ corresponds to 0·000153 gram of Mn_3O_4.

Results

1. *Estimation of manganese in the plant ash extract by the gravimetric and the bismuthate methods*

(*a*) It will be seen from the results given below in Table I that the amount of manganese determined by the gravimetric method is about eight times as great, although duplicate determinations carried out on different quantities of the stock solution by both the methods give results which agree fairly closely amongst themselves.

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* The nitric acid should be prepared from strong acid which has been freed from all traces of nitrous fumes by passing a current of air through it, which can be conveniently done with the help of a water pump.

(*) The asbestos used for this filtration is first digested with five per cent NaOH, washed with water, again digested with HNO_3 (1 : 4) and then washed until free of all traces of acidity. It is then dried, ignited and made into a suspension with wate .

TABLE I

Showing the amount of manganese obtained by the gravimetric and bismuthate methods

Volume of extract taken	Gravimetric method		Bismuthate method	
	Amount of manganese obtained as Mn_3O_4	Per cent Mn_3O_4 on oven-dried material	Amount of manganese obtained as Mn_3O_4	Per cent Mn_3O_4 on oven-dried material
25 c. c. .	0·0033	0·066	0·000428	0·0085
50 c. c. .	0·0067	0·067	0·000841	0·0084

(*b*) In order to test further, the relative accuracy of the two methods for estimating small quantities of manganese, an artificial plant extract containing known amount of manganese was prepared by adding small quantities of hydrochloric acid, ferric chloride, magnesium sulphate, calcium chloride, potassium phosphate and alum, etc. to a solution of manganese sulphate (0·7 grm. per litre). The figures for the manganese obtained by the two methods, are given below in Table II.

TABLE II

Determination of manganese in a solution containing known quantity of $MnSO_4$ in the presence of other salts

(10 c.c. of the solution=0·00354 grams of Mn_3O_4 were used for estimation)

Mn_3O_4 present in the solution	Gravimetric method		Bismuthate method	
	Amount of Mn_3O_4 as determined	Per cent of the the amount taken	Amount of Mn_3O_4 as determined	Per cent of the amount taken
0·00354	0·0060	170	0·00359	101·4
0·00354	0·0058	164	0·00359	101·4
0·00354	0·0056	158	0·00359	101·4

The results obtained by the gravimetric method are much higher than those by the bismuthate method which agree very closely with the amounts taken.

2. *Recovery of added manganese*

This was tested by adding to the extract known quantities of manganese in the form of *N*/100 potassium permanganate and deducting from the total amount determined the quantities present in the original extract as had been determined previously by the two methods respectively. The following results were obtained :—

TABLE III

Recovery of added manganese

Volume of plant extract taken for estimation.	Amount of manganese added as $N/100$ $KMnO_4$	Gravimetric method		Recovery per cent. of Mn_3O_4 added	Bismuthate method		
		Total Mn_3O_4 determined in the mixture	Mn_3O_4 recovered (after deducting the amount previously determined by this method)		Total Mn_3O_4 determined in the mixture	Mn_3O_4 recovered after deducting the amount previously determined by this method	Recovery per cent of Mn_3O_4 added
20 c.c.	2 c.c. = 0·000304 grms. Mn_3O_4	0·0060	0·0033	1085·5	0·000649	0·000312	102·6
25 c.c.	2·5 c.c. = 0·00038 grms. Mn_3O_4.	0·0088	0·0055	1447·5	0·000787	0·000359	94·5
25 c.c.	5 c.c. = 0·00076 grms. Mn_3O_4	...	...	...	0·001170	0·000750	98·7
25 c.c.	Do.	...	...	...	0·001200	0·000780	102·6
25 c.c,	Do.	...	...	...	0·001190	0·000770	101·3
50 c.c.	Do.	...	...	...	0·001553	0·000713	93·8

It is seen that the gravimetric method yields figures about ten times higher than those obtained by the bismuthate method. The average figure for the recovery of added manganese as obtained by the gravimetric method is, however, too high—about 1266 per cent *i.e.*, more than twelve times the quantity added, while in the case of bismuthate it is only ninety-eight per cent of the quantity added.

The higher results obtained by the gravimetric method seem to be partly due to incomplete precipitation of iron during the removal of phosphates leading to a small fraction of basic iron acetate escaping into the filtrate, with the result that this is precipitated along with manganese by bromine. Evidence of the fact that the precipitate is liable to be contaminated with iron was obtained, by fusing the ignited precipitate of Mn_3O_4 from a 10 c.c. solution of manganese sulphate with potassium bisulphate, dissolving the fused mass in hot water and determining the iron in this solution by colorimetric method using potassium sulphocyanide to develop the colour. But the amount of iron present as impurity in Mn_3O_4 as determined by the gravimetric method is not enough to explain the considerable difference in the amount of Mn_3O_4 as determined by this and the bismuthate method Evidently the precipitate must be contaminated with other substances besides iron. In order to obtain some knowledge of these impurities in the ignited Mn_3O_4 and also to trace other factors responsible for the discrepancies in the results obtained by the two methods, manganese was estimated in another set of solutions in the presence of potassium aluminium sulphate, aluminium phosphate, calcium chloride and calcium phosphate and the results obtained are given below :—

TABLE IV

Showing loss of Mn_3O_4 during the process of removal of phosphates, due to incomplete precipitation and the presence of impurities in the precipitate

Description	Amount per 100 c.c. of the extract					Mn_3O_4 carried away with iron-aluminium phosphates and basic iron acetate (4)
	Bismuthate method (1)	Gravimetric method (2)	Composition of Mn_3O_4 obtained by the gravimetric estimation (column 2) (3)			
			Mn_3O_4 determined by the bismuthate method (3a)	Iron as Fe_2O_3 (3b)	Other impurities (by difference) (3c)	
25 c.c. extract pot. alum. sulphate	0·00520	0·0144	0·00404	0·00100	0·00936	...
25 c.c. extract aluminium phosphate	0·00520	0·0312	0·00404	0·00463	0·02253	...
25 c.c. extract calcium chloride	0·00520	0·0120	0·00447	0·00018	0·00735	0·00037
25 c.c. extract calcium phosphate	0·00520	0·0120	0·00410	0·00125	0·00665	0·00061
25 c.c. extract . . .	0·00520	0·0080	0·00428	0·00034	0·00338	...
25 c.c. extract . . .	0·00520	0·0080	0·00441	0·00031	0·00328	...

As before the amount of manganese as found by the gravimetric method is much higher—varying from 150 to 600 per cent of that found by the bismuthate method and it would seem that the nature of the salts present in the solution, when bromine is added, exercises a considerable, though varying, influence on the amount of Mn_3O_4 finally obtained, and of the salts examined alumina seems to be the source of greatest error. In order further to test the purity of the ignited Mn_3O_4, the ignited precipitate obtained by the gravimetric method was fused with fused potassium bisulphate ; the fused mass after cooling was dissolved in hot water and made up to a definite volume. Manganese was determined in an aliquot portion by the sodium bismuthate method. The results obtained are given in column 3(*a*) of Table IV and approximate more closely to those obtained by the bismuthate method directly ; but they are now invariably lower. The cause of these low values seems to lie in the removal of a small amount of manganese with the iron-aluminium phosphates and basic iron acetate as is clear by the recovery of a fair amount of manganese from this precipitate in samples 3 and 4 (column 4). The sum of figures in columns 3 (*a*), and 4 is, however, still less than those in column 1, and these differences have been found to be due to the incomplete precipitation of manganese by bromine, the filtrate from which showed the presence of manganese by bismuthate method. Column 3 (*b*) of Table IV gives the amount of iron present as an impurity in the ignited Mn_3O_4 obtained by the gravimetric method. On further examination this solution was also found to contain traces of aluminium and phosphates. The inaccurate results obtained by the gravimetric method thus seem to be due to :—

(1) the removal of some manganese along with the iron-aluminium phosphate and basic iron acetate,

(2) the contamination of the final Mn_3O_4 precipitate with iron and aluminium phosphate, etc. and

(3) incomplete precipitation of manganese by bromine, whereby a small portion of manganese passes into the filtrate.

These results confirm Blum's observation [1912], that the composition of the precipitate in the gravimetric determination of manganese was not definite even under carefully regulated conditions. In addition to this main drawback, the gravimetric process is lengthy and tedious and requires almost continuous attention.

3. *Estimation of manganese as influenced by variations in temperature and acidity*

(*a*) *Vatiations of temperature.*—Blair [1904] has shown that the permanganic acid formed is not very stable in hot solutions and therefore recommends that the temperature of the solution before oxidation should be brought down to 15°C. In order to determine the range within which the temperature could be varied without affecting the accuracy of the results, determination of manganese was made in aliquot portions of the stock solution at different temperatures, with the results given below.

TABLE V

Estimation of manganese in plant material as influenced by the increase in temperature of the reacting solution

(Sample taken contained manganese equivalent to 0·0084 grm. of Mn_3O_4)

Temperature	Per cent Mn_3O_4 on oven-dried material.
10°C. . . .	0·0083
15°C. . . .	0·0083
20°C. . . .	0·0086
25°C. . . .	0·0083
30°C. . . .	0·0086
35°C. . . .	0·0061
40°C. . . .	0·0043

These figures indicate that the method is capable of giving accurate results at any temperature not exceeding 30°C.

(*b*) *Effect of variation in the concentration of the acid.*—Before proceeding with the oxidation of manganese with 4 *N* nitric acid, it is necessary to use a small amount of concentrated sulphuric acid in order to remove all traces of hydrochloric acid from the residue obtained by the evaporation of the hydrochloric acid extract of the plant ash. In order to remove all excess of sulphuric acid the contents must first be heated to dryness and then ignited. The process is not only lengthy but is also attended with the risk of loss of material due to decrepitation during ignition. In order therefore to determine the amount of sulphuric acid which could be allowed to remain behind after the complete removal of hydrochloric acid, aliquot portions from the stock solution were taken and after the addition of 5 c.c. of sulphuric acid some of them were dried to the stage of production of white fumes of SO_3; while others were evaporated to complete dryness. The results given below indicate that this precaution is superfluous and the presence of sulphuric acid to the extent of 5 c.c. per 50 c.c. of 4 *N* nitric acid does not introduce any inaccuracy.

TABLE VI

Effect of the presence of concentrated sulphuric acid in varying amounts on the oxidation of manganese with 4 N *HNO_3*

(5 c.c. concentrated sulphuric acid were used in each case)

Volume of extract taken	Percentage amount of Mn_3O_4 determined	
	Sulphuric acid evaporated to dryness and ignited	Sulphuric acid evaporated to the stage when white fumes of SO_3 are produced
20 c. c.	0·0085	0·0084
25 c. c.	0·0084	0·0085
50 c. c.	0·0083	0·0084

The process of ignition to remove excess of sulphuric acid would thus seem to be quite unnecessary.

Table VII gives the amount of manganese found by taking different quantities of the same plant extract and it will be seen that the amounts of manganese per 100 grams of the oven-dried material calculated from all these determinations agree very closely with their mean value ($\chi^2=1{\cdot}619$, $P>0{\cdot}9$).

TABLE VII

Volume of extract taken	Total Mn_3O_4 found	Per cent Mn_3O_4 on oven-dried material
20 c.c.	0·000342	0·0085
20 c.c.	0·000337	0·0084
25 c.c.	0·000428	0·0085
25 c.c.	0·000421	0·0084
25 c.c.	0·000413	0·0083
25 c.c.	0·000413	0·0083
25 c.c.	0·000428	0·0086
25 c.c.	0·000413	0·0083
25 c.c.	0·000428	0·0086
50 c.c.	0·000834	0·0083
50 c.c.	0·000841	0·0084

Mean = 0·00842 ±0·000111

The standard error of difference in the amount of maganese found by parallel estimations is so small that the bismuthate method for the determination of manganese can be relied upon to give excellent results under sufficiently wide range of conditions provided the precautions necessary for its estimation are properly observed.

In Table VIII are given the results of the estimation of manganese in the same set of six samples of fodders analysed by different workers using their own different methods. Samples No. 2 and 3 are duplicates of Nos. 6 and 5 respectively and were prepared from the same material, but this fact was not known to the analyst at the time of analyses. The set of figures given under gravimetric method (*a*) was obtained at the Chemical Research Laboratories of the Agricultural Chemist to Government Dacca, Bengal and that given under (*b*) at Mandalay, Burma. It will be seen that the gravimetric method not only gives higher figures than those obtained by the bismuthate and the periodate methods, but that in the hands of two different workers, the results obtained are liable to differ widely. The figures for the amount of manganese obtained with the bismuthate and the periodate methods show a fairly close agreement.

TABLE VIII

Relative values for the amount of manganese in different remples of fodders, as determined by the bismuthate, periodate and gravimetric methods

Bismuthate method	Periodate method	Gravimetric method	
		(a)	(b)
1. 0·009	0·008	0·003	0·407
2. 0·011	0·012	0·018	0·139
3. 0·006	0·008	0·009	0·282
4. 0·014	0·016	0·022	0·362
5. 0·007	0·008	0·027	0·075
6. 0·009	0·012	0·018	0·318

It is clear that the gravimetric method gives much higher figures, which are not identical in the hands of two different workers; whereas the results obtained by the bismuthate and the periodate methods agree fairly closely.

SUMMARY

1. The gravimetric method for the estimation of manganese very often gives abnormally high values.

2. As judged by the concordance of results obtained with parallel estimations and the recovery of added manganese to the hydrochloric acid extracts of plant ash, the sodium bismuthate method for the volumetric determination of manganese in plant materials has been found to give excellent results.

3. In solutions containing different salts approximating in composition to the plant extract, the amount of manganese determined by this method agrees very closely with the amount known to be present.

4. For obtaining accurate results it is necessary (1) to use nitric acid free from all traces of nitrous fumes and (2) to remove every trace of hydrochloric acid before oxidation.

5. Temperature of the solution before and after oxidation must not exceed 30°C.

6. It is not necessary to remove sulphuric acid before carrying out the oxidation.

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UTILIZATION OF CERTAIN FORMS OF INORGANIC NITROGEN DURING THE DECOMPOSITION OF PLANT MATERIALS IN THE SOIL

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(Received for publication on 29th October 1935)

Although it is known that the adverse effect consequent on the decomposition of stubble can be largely remedied by liberal application of nitrates or ammonium salts, it is yet not clear as to which of these two forms is preferentially utilized by micro-organisms. A study in this direction has, however, been made by Richards and Shrikhande [1935] who found a preference for the ammoniacal over the nitric nitrogen in the early stages while working with plant products alone. A similar study in another direction has been made by Shive and co-workers [1933] by following the relative rates of absorption of ammoniacal and nitric nitrogen by plants grown in culture solutions. They observed greater ammonia absorption in the early stages than nitrate. The latter was absorbed to a maximum in the later stages. The reactions were largely controlled by the pH of the culture solution.

The phenomenon of the decomposition of plant residues in the soil in presence of different forms of inorganic nitrogen is more complicated than the study of decomposition of plant products alone. Such a study in the soil involves the consideration of at least four factors :—(*i*) absorption of ammonia by soil colloids, (*ii*) movement of the mineral forms of nitrogen in the soil depending upon its physical structure, (*iii*) nitrification of the decomposed organic matter initially present in the soil and losses of nitrogen through de-nitrification and (*iv*) assimilation of soluble mineral nitrogen by the micro-organisms in the soil to elaborate their tissue materials. The following investigation was, therefore, undertaken to study, under laboratory conditions, the relative rates of utilization of nitrogen in the ammoniacal and nitric forms by the micro-organisms concerned in the decomposition of plant tissues when both forms are almost equally available by following periodically the changes in ammoniacal, nitric and total nitrogen. The experiments were so arranged as to take into account the above-mentioned possible reactions going on in the soil. Two soils—one wet cultivated and the other dry cultivated—were used in the present study.

EXPERIMENTAL

The soils were air-dried and screened through a one-mm. sieve. Finely powdered *ragi* (*Eleucine coracana*) straw was added at the rate of three tons to an acre. Hundred-gram lots of soil with 0·3 grm. each of straw passing through one-mm. mesh sieve, after thoroughly mixing, were placed in wide-mouthed bottles of 250 c.c. capacity. Ammonium sulphate and sodium nitrate were added in aqueous solution at the rate of one grm. of soluble nitrogen per 100 grms. of straw, approximately equivalent to the nitrogen factor [Hutchinson and Richards, 1921], *i.e.*, each bottle was supplied with 0·003 grm. (30 p. p. m.) of soluble nitrogen either wholly as ammonium sulphate or sodium nitrate, or 0·0015 grm. of each where an equivalent mixture of both was added. The moisture content was brought to about twenty per cent, the mixture well-stirred and the bottle plugged with cotton-wool. Loss of moisture was made up by adding water from time to time. Separate samples were incubated at 30°C. for the different periods of decomposition. Five sets of bottles containing five each for each soil were incubated. The analysis for each set was carried out at intervals of three, seven, fourteen, twenty-eight and fifty-six days, as was done in the case of pure plant products [Richards and Shrikhande, 1935]. Four sets with each soil contained straw while the fifth one was left untreated as control.

One bottle was removed at each stage and the following determinations were made on its contents :—

Ammoniacal nitrogen.—This was estimated in the potassium chloride extract at pH 1·0 as described by Ölsen [1929] by distillation with magnesia.

Nitric nitrogen.—This was determined on the residue after ammonia-nitrogen distillation by reduction with Devarda's alloy under alkaline conditions.

Total nitrogen.—This was estimated by the usual Kjeldahl method using sulphuric-salicyclic acid mixture [A. O. A. C., 1930].

Analytical figures for the soil nitrogen and the nitrogen content of straw are contained in Table I.

TABLE I

Analytical figures for the soil and straw (expressed as parts per million)

—	Wet cultivated soil	Dry cultivated soil
Total nitrogen (soil)	174	720
NH_3-N	4	4
NO_3-N	2	9
N in the straw added	16	16
NO_3-N+NH_3-N added	30	30
∴Total N	220	766
Organic N	184	723

Table II deals with the figures for the different forms of nitrogen obtained experimentally and have been expressed as parts per million. The results for both the soils have been included in the same table to facilitate comparison. Section (A) deals with wet cultivated soil alone ; (B) with soil+straw ; (C) with soil +straw+equal amounts of ammoniacal and nitric nitrogen ; (D) with soil+straw+ammoniacal nitrogen ; and (E) with soil+straw+nitric nitrogen ; the corresponding sections with dashes represent the figures for the dry cultivated soil.

TABLE II

Forms of nitrogen in parts per million (figures actually obtained)

Days	Wet cultivated soil				Dry cultivated soil			
	NH_3-N	NO_3-N	Total N	Organic N	NH_3-N	NO_3-N	Total N	Organic N
	Soil alone (A)				*Soil alone (A′)*			
3	4	..	189	185	13	15	721	693
7	11	3	180	166	9	15	711	687
14	10	8	164	146	12	16	700	672
28	12	14	165	139	11	22	716	683
56	10	16	166	140	10	30	718	678
	Soil+Straw (B)				*Soil+Straw (B′)*			
3	1	2	202	199	2	3	750	745
7	1	3	196	192	1	2	749	746
14	1	2	19	190	2	2	744	740
28	1	2	..	180	1	1	..	739
56	1	2	198	195	1	1	751	749
	Soil+Straw+NH_3-N+NO_3-N (C)				*Soil+Straw+NH_3-N+NO_3-N (C′)*			
3	17	25	..	233	24	33	724	667
7	14	18	215	183	12	33	744	699
14	12	6	215	197	13	26	755	716
28	6	6	215	203	10	31	755	714
56	11	..	202	177	9	21	760	730
	Soil+Straw+NH_3-N (D)				*Soil+Straw+NH_3-N (D′)*			
3	20	11	244	213	37	21	757	699
7	17	8	228	203	17	14	751	720
14	12	3	215	200	10	16	750	724
28	6	2	216	208	9	20	754	725
56	15	2	205	188	10	18	..	742
	Soil+Straw+NO_3-N (E)				*Soil+Straw+NO_3-N (E′)*			
3	8	36	241	197	13	41	757	703
7	9	30	191	151	14	26	745	705
14	11	18	202	173	13	19	..	737
28	11	9	213	193	19	21	760	720
56	12	16	216	187	9	31	748	708

Considering the case of soil alone (sections A and A′), the ammoniacal nitrogen remains practically stationary for both the types of soil, whereas there is a gradual increase in the amount of nitric nitrogen with the period of incubation, suggesting the presence of decomposing organic matter in the soil. Evidence to this effect was also obtained while treating the soil with hydrogen peroxide during the course of mechanical analysis. The difference between the amount of nitrate on the 3rd and 56th days is practically of the same order (about 15 p. p. m.) in both the soils. When straw is incorporated into the soil (Section B and B′) there is considerable fall in the values for both the ammonia and nitrate. These lower values are no doubt due to the assimilation of both the forms by the micro-organisms during the decomposition of the straw. The utilisation of ammoniacal nitrogen is usually complete, partly due to its absorption by the soil colloids and partly to its assimilation by the micro-organisms for their structural development, thus allowing little or none for losses due to volatilisation. But where nitric nitrogen is involved, it is necessary to consider that the gradual fall in nitrate may be due not only to assimilation by the micro-organisms but also to loss as elementary nitrogen, although it would appear from an examination of the value for total nitrogen that this latter is negligible.

Surveying the results as they appear in Table II, without accounting for the control, the following observations can be made :—

Section (*C and C′*).—In the wet cultivated soil when ammonia and nitrate have been added in equal amounts, the quantity of ammonia is practically constant, whereas there is a heavy drop in the nitric nitrogen as the period of incubation increases. With dry cultivated soil under identical conditions the reverse is the case.

Section (*D and D′*).—The addition of ammonia nitrogen to the soil and straw mixtures appears to result in a rapid fall of the ammonia content of the dry cultivated soil with time, whereas with the wet cultivated one the change is not so marked. The changes in the nitrate content are the reverse of the above. It is possible that in the dry cultivated soil the decrease in the ammonia content may be due to assimilation by micro-organisms since the figure for total nitrogen remains practically constant. The greater preference of ammonia in the dry cultivated soil may also be accounted for by considering the relative rates of movement of ammonia and nitrate in the soil. It is known that nitrate moves down faster than ammonia in a light soil, thus removing it from the reach of the organisms. They have, therefore, to seek recourse to what is readily available. The movement of the two forms of nitrogen is dealt with separately. The constant values for the nitrate in the dry cultivated soil may be more apparent than real because of the nitrification of the decomposed organic matter initially present in the soil.

Section (*E and E′*).—The addition of nitrate to the soil treated with straw does not bring about any marked variation in the nitrate content of the dry cultivated soil, but in the wet cultivated one a rapid fall in the nitrate content is observed. A comparison with the figure for total nitrogen in the latter case shows that this fall in the nitrate content may possibly be due to loss by de-nitrification.

By subtracting the figures for ammoniacal and nitric nitrogen from the total nitrogen present in a particular sample, an estimate of the organic nitrogen present in it can be made. It will be observed in all the three treatments mentioned above, that both the total and organic nitrogen are almost of the same order with the dry cultivated soil as those initially present. With the wet cultivated soil, however, it is difficult to state with the available data whether nitrogen is not lost by denitrification and probably also due to volatilisation as ammonia, with the result that there is usually a fall in all the cases of the organic nitrogen.

The above observations are more clearly brought out from the results included in Table III where allowance has been made for values obtained with the control experiments.

TABLE III

Figures expressed in parts per million after accounting for the figures in the control experiments

Days	Wet cultivated soil		Dry cultivated soil	
	NH_3-N	NO_3-N	NH_3-N	NO_3-N
	Effect of straw on the soil NH_3 and NO_3 nitrogen			
	(*A*)		(*A′*)	
3	3	..	11	12
7	10	..	8	13
14	9	6	10	14
28	11	12	10	21
56	9	14	9	29
	Soil+Straw+NH_3-N+NO_3-N			
	(*B*)		(*B′*)	
3	16	23	22	30
7	13	15	11	31
14	11	4	11	24
28	5	4	9	30
56	10	..	8	20

TABLE III—*contd.*

Days	Wet cultivated soil		Dry cultivated soil	
	NH_3-N	NO_3-N	NH_3-N	NO_3-N
	Soil+Straw+NH_3-N			
	(*C*)		(*C'*)	
3	19	9	35	18
7	16	5	16	12
14	11	1	8	14
28	5	..	8	19
56	14	..	9	17
	Soil+Straw+NO_3-N			
	(*D*)		(*D'*)	
3	7	34	11	38
7	8	27	13	24
14	10	16	11	17
28	10	7	18	20
56	11	14	8	30

Section (*A and A'*).—The ammonia is practically the same as in the soil alone (Table II, Sections A and A'), whereas there is a difference between the nitrate content of the soil alone and when straw is introduced into it. There is, however a greater variation in the nitric nitrogen in wet cultivated than in the dry cultivated soil. The almost steady figures for nitrate in the dry cultivated soil may be ascribed to the greater amount of nitrification for which the conditions are more favourable due to its coarse texture and consequently greater aeration.

Section (*B and B'*).—The absorption of nitrate in the wet-cultivated soil is double that of ammonia between the 3rd and the 7th day and nearly five times as much between the 7th and the 14th day. In the dry cultivated soil nitrate is practically constant throughout the period of incubation while ammonia is heavily used up. These observations show that the organisms have a preference for ammonia in the dry cultivated soil whereas in the wet cultivated soil the low recovery of nitrate might either mean a preferential utilization by the organisms or a loss of nitrate due to de-nitrification. The latter explanation seems to be more probable because of the low values obtained for the total nitrogen wherever nitrate has been added. Further work is needed to establish either of the two possibilities.

Section (*C and C'*).—Here more than thirty per cent of the total ammonia has been used up in the first three days in the wet cultivated soil, the absorption getting slower as the period increases. In the dry-cultivated soil however, ammonia absorption is practically negligible in the first three days and maximum in the next ten days. This apparently contradictory result for the wet cultivated soil can be accounted for by considering the abundance of ammonia which is more than double of that present in section (B and B′). The relatively higher figure for nitrate in the dry cultivated soil may be due to the conversion of some of the ammonia into nitrate during diffusion as pointed out by Cook [1916] and also due to nitrification.

Section (*D and D′*).—Ammonia is almost constant and nitrate is utilised in both the soils, the maximum absorption being in the earlier stages in the dry cultivated soil and in the wet cultivated in the later stages. Such a difference may be accounted for by the relative rates of movement of the two forms of nitrogen in the two different soils.

Relative movements of ammonia and nitrate nitrogen in the soil

That there is a difference in the relative rate of downward movement of the two forms of nitrogen depending upon the physical structure of the soil has been pointed out by several workers.

Karpizov [1904] in investigating the absorptive capacity of different soil layers, found it at a maximum in the surface layers decreasing directly with the humus and zeolites towards the sub-soil. The degree of absorption varied with the mechanical composition and the hygroscopic moisture content of the soil. Sabanin [1908] confirmed the observations of Karpizov. Zhottsinski [1908] working with Russian soils noted a relationship between the mechanical composition and ammonia absorption. Cook [1916] while studying the retention and distribution of ammonium sulphate found that ammonia moves down quicker to lower layers in the light but in the heavy soils most of the ammonia is held in the top surface of three or four inches. The experience of these workers indicates that the movement of ammonium sulphate in the soil is slow and actual rate varies with the nature of the soil. Nitrogen in the nitric form is more easily diffused than nitrogen in any other form as has been pointed out by several workers.

To study on a small scale whether these observations hold good for the experiments described in this paper both the types of soils were filled in to a height of nine inches in cylinders of 9-in. height and 2½-inch diameter made of paraffined paper. The cylinders were made to stand in glass dishes of about the same diameter. To give a greater stability for the cylinders they were placed in a wooden chest with frame for each. The soil which weighed about 600 grms. was brought to a moisture content of twenty per cent and then poured into the cylinder. Nitrogen in the form of solution of ammonium sulphate and sodium nitrate to the extent of 0 05 grm. was poured on the soil surface. Cylinders thus made facilitate cutting the soil columns at a particular depth with any metal or glass plate preventing any mixing of the soil with other layers.

The usual depth of surface soil is reckoned as top 9 in. and hence the particular height of the soil column in this experiment. The cylinders were cut at depths of 3 in. and 6 in. from the top and the amount of ammonia and nitrate estimated at intervals of three, seven and thirty days. The results obtained are included in Table IV. They agree with the observations of foregoing workers. The movement of ammonia is greater in the dry soil than in the wet one. There is also an increase in the nitrate in different layers in the red soil due partly to the conversion of ammonia into nitrate but mostly to the nitrification of the decomposed organic matter initially present. In the paddy soil, however, nitric nitrogen is practically constant. The greater movement of nitrate in the red than in the paddy soil can clearly be seen by comparing the drop from 0·051 grm. to 0·043 grm. in the former with 0·045 grm. to 0·043 grm. in the latter between the 3rd and the 7th day. The comparatively high value of 0·044 grm. nitrate in the red soil on the 30th day must be due to the nitrification of the organic matter. This fact is also borne out by the increasing amounts of nitrate in the different layers and also by the progressive nitrate production during the incubation of soil alone.

Table IV

NH^3 and NO_3 nitrogen content of different soil layers during the movement of the two forms of nitrogen in the soil

(*Figures in grm. actually obtained in the experiment*)

Days	Top 3-in. layer		Middle 3-in. layer		Bottom 3-in. layer	
	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3
	Dry cultivated soil+0·05 grm. NH_3-N					
3 . .	0·050	0·003	0·005	0·004	0·003	0·002
7 . .	0·043	0·003	0·003	0·003	0·003	0·003
30 . .	0·040	0·008	0·002	0·001	0·001	0·003
	Wet cultivated soil+0·05 grm. NH_3-N					
3 . .	0·046	0·001	0·001	..	0·008	..
7 . .	0·044	0·001	0·001	..	..	..
30 . .	0·044	0·001	0·001	..	0·001	..
	Dry cultivated soil+0·05 grm. NO_3-N					
3 . .	0·003	0·051	0·003	0·003	0·003	0·003
7 . .	0· 02	0·043	0·002	0·003	0·00	0·003
30 . .	0·002	0·044	0·001	0·004	0·001	0·004
	Wet cultivated soil+0·05 grm. NO_3-N					
3 . .	..	0·045	..	0·003	..	0·001
7 . .	..	0·043	..	0·005	..	..
30 . .	0·001	0·040	..	0·007	..	..

DISCUSSION

As a result of these studies a few observations can be made with regard to the selection and application of a nitrogenous fertilizer to the soil. The salt used as fertilizer splits up almost immediately into ions. The cations are absorbed by the soil colloids and the anions liberated. Ammonia from ammonium sulphate is absorbed by the soil colloids, and may later become available for the structural development of the micro-organisms. In the case of sodium nitrate, however, the sodium, after splitting will be absorbed by the soil complex letting the nitric nitrogen free which becomes available to the organisms and the plant. The results of the present experiments have shown that in the dry cultivated soil ammoniacal fertilizer in conjunction with organic manures might prove useful in the conservation of soil organic matters. But as stated previously it is difficult to say with the available evidence as to what may happen when nitrates are added to the soil especially in the wet cultivated one. Another factor which plays an important part in fertilising practice is the movement of soluble forms of nitrogen in the soil. This movement, of course, depends upon the nature of the soil as evidenced by several workers. In the wet cultivated soil ammonia is absorbed by the colloids leaving little for the downward movement whereas the nitrate is free to move about. In the dry cultivated soil such a movement of both the ions is facilitated by the deficiency of soil colloids as also by greater pore space on account of the coarser nature of the soil praticles. The nature of the soil should, therefore, be a guide in the application of a soluble nitrogenous fertilizer. In the heavy soil nitrate ought to be a better fertilizer or a supplement when organic matter is added or a crop sown. It may be applied either as nitrate of chalk or ammonia rather than as sodium nitrate which might produce an adverse effect on physical the structure of the soil. Moreover it will have less chance of leaching here than in the light soil. In the light soil, ammonia should be better adapted as it will be more easily available than in the heavy. As nitrate moves faster than ammonia in the light soil it would be more economic to apply a dressing of ammonia than nitrate which, on account of its greater downward movement and solubility, may be washed down and removed from the zone of activity of the plant and the micro-organisms.

SUMMARY

(1) Incorporation of straw with soil depresses the amount of ammoniacal and nitric nitrogen present in the soil.

(2) When straw with an almost equal dressing of ammonium sulphate and sodium nitrate are introduced into the soil, there is a definite preference by the micro-organisms concerned in the decomposition for ammoniacal nitrogen in the dry cultivated soil over nitric nitrogen whereas reverse may be the case in the wet cultivated soil. Further work is needed to establish the preferential utilisation in the wet—cultivated soil.

(3) Nitrate appears to move down faster than ammonia in both the soils. Compared amongst themselves, ammonia goes down quicker in the dry cultivated than in the wet cultivated soil. The rate of movement is rather slow.

(4) Practical applications of the results obtained have been discussed.

ACKNOWLEDGMENT

The author's thanks are due to Professor V. Subrahmanyan, for offering the facilities of the Department and criticising the manuscript.

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ON THE NATURE OF NITRIFICATION IN SOIL

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(Received for publication on 25th November 1935)

Nitrification in soils has been generally accepted to be a bacterial process. Recently Dhar, Bhattacharya and Biswas [1933] have suggested that nitrification in tropical soils is more photo-chemical than bacterial in nature. Rossi [1933] also believes that rapid nitrification in soil can take place by purely physico-chemical process.

Sarkaria and Fazal-ud-Din [1933] tested the photo-catalytic power of some Punjab soils, and found that the soils showed only a weak catalytic action. Similar results were obtained by Fraps and Sterges [1935] with some American soils.

In view of the great importance of nitrification in soils, experiments were undertaken to find out whether photo-nitrification or bacterial nitrification predominated in the Punjab soil.

Methods of analysis

Nitrous nitrogen. By Griess Illosvay reagent.

Nitric nitrogen. By Phenol-disulphonic acid.

Chlorides, whenever present in excess, were completely removed by precipitation with silver sulphate before treatment with the reagent.

Experimental

Freshly air-dried soil was passed through one mm. sieve and mixed with ammonium phosphate [*$(NH_4)_2HPO_4$] at the rate of fifty-seven mgs. of nitrogen per 100 grams of soil. The treated soil was put in nine glass jars at the rate of 1800 grams per jar. The jars were divided into three sets of three each. In each set, soil of one jar was mixed with nine grms. of mercuric chloride to eliminate all living organisms, the second was covered with a metallic lid to prevent access of light and the third was left uncovered.

In the first set, the soil was kept air-dry, in the second, at thirteen per cent moisture content, and in the third, at thirty-nine per cent moisture content. All the jars were wrapped with black paper on the outside and exposed to the sun. Loss in moisture was made up from time to time. The experiment was started on 28th March. Samples were analysed after 65 days and 100 days. The results are given in Table I.

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* According to Dhar and others [1933] ammonium phosphate was found to be more rapidly nitrified than any other compounds of ammonia.

TABLE I

Nitrification of ammonium phosphate in soil

(Mgs. of nitrogen per 100 grms. of soil)

Treatment		65 days			100 days		
		Nitrous N	Nitric N	Percentage oxidised	Nitrous N	Nitric N	Percentage oxidised
Air-dry	Sterilized and exposed.	Trace	4·5	0·2	Trace	7·7	5·7
	Unexposed .	,,	4·8	0·7	,,	6·0	2·8
	Exposed .	,,	4·8	0·7	,,	7·5	5·4
13 per cent moisture.	Sterilized and exposed	Trace	4·6	0·4	Trace	6·3	3·3
	Unexposed .	,,	11·6	12·6	,,	12·0	13·3
	Exposed .	,,	7·2	4·9	,,	9·5	8·9
39 per cent moisture.	Sterilized and exposed	Trace	4·0	...	Trace	4·4	0·0
	Unexposed .	,,	4·0	...	,,	4·4	...
	Exposed .	,,	3·2	...	,,	1·5	...

Initial { Ammoniacal nitrogen . . . . . 57·0 mgs.
Nitrous nitrogen . . . . Trace
Nitric nitrogen . . . . 4·4 mgs.

It will be seen from the above that :—

(1) under air-dry condition of the soil, nitrification was insignificant either by biological or photo-chemical means during first sixty-five days, while on exposure to 100 days, 5·8 per cent of the added nitrogen was converted into nitrate in presence of light as compared with 2·8 per cent in the absence of light.

(2) At optimum moisture content no nitrification took place by photo-chemical process but 12·6 per cent nitrified by the biological process during first sixty-five days, and in 100 days the photo-chemical nitrification was 3·3 per cent as compared to 13·3 per cent by the biological means.

(3) Under saturated moisture contents no photo-nitrification took place even after 100 days' exposure, and nitrogen was reduced rather than oxidised by the biological process.

Very low nitrification by the biological process, during the experiment, was found to be due to the temperature of the soil, which was very high (above 48°C.) and inhibitory after the first fortnight of the experiment.

Fazal-ud-Din [1935] noticed that the catalytic action of the soil was different for different nitrogenous compounds. In order to form a correct estimate of photo-nitrification as compared to biological nitrification, it was thought necessary to try the nitrification of a number of compounds by both the processes.

Fresh, air-dried soil was passed through one mm. sieve and different nitrogenous compounds were added to supply forty mgs. nitrogen per 100 grms. of soil. In the first series, the moisture was made up to thirteen per cent by saturated mercuric chloride solution, while in the second, it was made up by distilled water. The soils so prepared were taken in 200 grm. portions in duplicate conical flasks of 500 c.c. capacity . The flasks were plugged with cotton wool and in the first series they were exposed to the sun, while in the second series, they were wrapped in black paper and kept side by side.

As the experiment was intended to run for three months, the loss of moisture had to be made up frequently, therefore sterilization of the soil by treatment with mercuric chloride was preferred to steam sterilization to avoid a chance contamination with nitrifying bacteria and consequent error.

The experiment was started on 12th January and terminated on 12th April, when the soils were analysed for nitrous and nitric nitrogen. The results given in Table II indicated that the different nitrogenous compounds were nitrified to varying degrees (from 36·0 to 64·7 per cent) by the biological process, while under the same conditions no nitrification took place by photo-chemical means.

TABLE II

Nitrification of different nitrogenous compounds in soil

(Mgs. of nitrogen per 100 grms. of soil)

Nitrification of	Photo-nitrification		Bacterial nitrification		
	Nitrous N	Nitric N	Nitrous N	Nitric N	Percentage oxidised
Ammonium chloride .	Trace	3·1	0·2	17	36·0
Ammonium sulphate .	,,	3·1	0·3	21	46·2
Ammonium acetate .	,,	2·5	0·2	28·5	64·7
Ammonium oxalate .	,,	2·5	0·2	26·5	59·7
Glycocoll . . .	,,	2·3	0·4	19	41·5
Acetamide . . .	,,	2·5	0·3	19	41·2
Creatinine . . .	,,	2·5	0·2	20	43·5
Urea	,,	2·6	0·2	23	51·0
Uric acid . . .	,,	2·9	0·2	20	43·5

Initial	Nitrous nitrogen	Trace
	Nitric nitrogen	2·8 mgs.

The absence of photo-nitrification in the above series suggested the possibility of the inhibitory action on the process by mercuric chloride treatment. Although mercuric chloride did not show any poisoning effect on the catalytic action of the soil in the previous experiments, nevertheless, it was of interest to investigate its effect on the action of a powerful catalyst.

One hundred c.c. of $N/2$ ammonium chloride solution and one gram of zinc oxide were taken in each of the four beakers of 400 c.c. capacity. Two of these beakers were treated with half a gram of mercuric chloride . These were exposed to the sun and washed air was slowly bubbled through each at approximately the same rate. Nitrous nitrogen was estimated after six hours. The results given below showed that mercuric chloride did not poison the catalyst.

Treatment	Mgs. of nitrous nitrogen per 100 c.c. of the solution	
Without mercuric chloride	0·26 0·22	0·24
With mercuric chloride	0·22 0·19	0·20

In the case of mercuric chloride treated solutions, oxidation was slightly reduced. This was partly due to the suspension becoming slightly greyish and partly due to the reduced surface of the catalyst because of the conglomeration of the fine particals of zinc oxide. However, the effect was only slight.

Experiments were undertaken to find out how much increase in photo-nitrification was obtained by incorporation of a small amount of a powerful catalyst with soil. For this purpose two per cent zinc oxide was mixed with some soil. Ammonium sulphate was added at the rate of forty mgs. of nitrogen per 100 grams of soil and the mositure content made up to thirteen per cent. Two hundred and eighty grams of the prepared mixture was equally distributed into four flasks of 300 c.c. capacity. Four control flasks were similarly set up without zinc oxide. The flasks were plugged with cotton wool and sterilized at 120°C. for one hour on two consecutive days. The plugs were covered with paper. The flasks were exposed to the sun and analysed after a month for nitrous and nitric nitrogen. There was no difference in the nitrate contents of the flasks. There was, however, some difference in the nitrite contents as shown below :—

Treatment	Mgs. of nitrous nitrogen per 100 grms. of soil	
Soil + zinc oxide	0·44 0·46 0·44 0·44	0·44
Soil alone	0·22 0·24 0·20 0·13	0·20
Difference		0·24

This showed that the catalytic power of the soil could be increased by the addition of a powerful catalyst but not to any great extent.

Dhar and co-workers found that ammonium phosphate was almost completely nitrified photo-chemically in three months' time. Compared to that the photo-nitrification of ammonium phosphate in Lyallpur soils was very small i,e., 5·7 per cent. The soils of the Punjab were found to be weak photo-catalysts [Sarkaria and Fazal-ud-Din, 1933], therefore nitrification in these soils appeared to be mainly biological in nature. In summer the rate of photo-nitrification was very small while in winter no photo-nitrification took place.

As regards the high nitrate content of the surface soils during summer months which has been attributed by Dhar and others as being due to the active photo-oxidation, it might be explained by the rise of nitrates from lower depths due to the increased rate of evaporation during summer months. Bacterial nitrification during summer is inhibited on the surface on account of the high temperature, low moisture contents, and sterilizing action of the rays. But nitrification goes on at lower depths where conditions are more favourable. Thus it would be apparent that the process of nitrification is mainly a biological one in the tropical soils of the Punjab, and the small amount of oxidation by photo-chemical process which can take place only in summer, is of very small importance.

After studying the oxidation of various nitrogenous compounds in soil, some experiments were undertaken to study the comparative rates of nitrification in solution by the two processes.

Fifty c.c. of dilute (1 : 3) Omeliansky solution less ammonium sulphate with one per cent calcium carbonate were put in conical flasks of 300 c.c. capacity. Various nitrogenous compounds were separately added in sets of four flasks to supply five mgs. of nitrogen per flask and sterilized. Volatile compounds were added after sterilization. Each flask was inoculated with one gram of soil, wrapped in black paper and kept over roof.

Another series of seventy-two flasks was divided into two sets. In one set, one gram of zinc oxide, and in the other five grams of soil were added in each flask. The same nitrogen compounds as used in the first series were added at the same rate to the four flasks of each set and the volume was made up to fifty c.c. with distilled water, sterilized and kept over roof. Both the series were started together in February. The contents of the flasks were analysed after two, three and four weeks. The results are given in Table III. The results of oxidation in presence of soil catalyst have been omitted as the oxidation was negligibly small.

Photo-oxidation with zinc oxide as a catalyst was more than bacterial oxidation during the first two weeks. During the third week, the oxidation by bacteria increased and finally in the fourth week, it was forty-seven to ninety-seven per cent. Maximum photo-oxidation after four weeks was only nineteen per cent.

It was found that the rate of multiplication of nitrifying bacteria was very slow in Omeliansky solution and therefore the rate of oxidation of nitrogen is slow in the beginning, later on when sufficiently large number of organisms are present the rate is very swift and even a few hours suffice for the appreciable oxidation of the nitrogen added as ammonium sulphate.

TABLE III

Nitrification in solution

(Mgs. of nitrogen per 100 c.c. of solution)

Nitrification	Bacterial						Photo (zinc oxide as catalyst)					
	Two weeks		Three weeks		Four weeks		Two weeks		Three weeks		Four weeks	
of	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N	Nitrous N	Nitric N
Am. hydroxide	0·1	0·1	0·4	0·2	6·8	0·2	Trace	0·8	Trace	1·2	Trace	1·6
Am. sulphate	0·4	0·1	7·8	0·6	9·1	0·3	,,	1·0	,,	1·2	,,	1·9
Am. carbonate	0·4	0·1	4·9	0·3	5·5	0·3	,,	0·6	,,	1·5	,,	1·4
Am. acetate	0·3	0·3	3·2	0·2	6·2	0·2	,,	0·3	,,	..	,,	0·8
Am. oxalate	0·1	0·1	0·3	0·1	6·5	0·2	,,	1·1	,,	1·9	,,	1·4
Glycocoll .	0·1	0·1	0·1	0·1	4·5	0·2	,,	0·5	,,	0·6	,,	1·1
Acetamide .	0·1	0·1	0·7	0·1	6·8	0·2	,,	0·3	,,	0·5	,,	0·9
Creatinine .	0·2	0·1	2·5	0·2	4·5	0·4	0·1	0·6	,,	0·6	,,	0·8
Urea .	0·3	0·1	1·7	0·2	0·1	9·6	Trace	0·6	,,	0·5	,,	1·1

Initial		
	Nitrous nitrogen . .	trace
	Nitric nitrogen . .	0·3

In a number of 300 c.c. flasks, twenty c.c. of dilute Omeliansky solution was inoculated with six c.c. of actively nitrifying Omeliansky solution and incubated at 35°C. It was found that about one milligram of ammoniacal nitrogen was oxidised in six hours.

TABLE IV

Rate of nitrification in Omeliansky solution

Incubation period (hours)	Mgs. of nitrous nitrogen per 100 c.c. of solution
Initial	2·75
12	3·89
18	4·86
24	5·83
36	7·78
42	9·31

This showed that in an actively nitrifying solution the rate of oxidation by bacteria is many times more than the oxidation by the sun. If however, the medium is not favourable for the growth of nitrifying bacteria, then bacterial nitrification becomes negligible in comparison to oxidation by solar radiations as has been shown elsewhere [Fazal-ud-Din, 1935].

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STUDIES IN THE GERMINATION OF CROP SEEDS IN THE FIELD

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(Received for publication on 7th January 1936)

(With two text-figures)

I. Introduction

The importance of good stand in a crop can hardly be over-emphasized. Too thick or too thin a stand always results in a low yield and consequently makes farming less remunerative. The need for a good stand is equally important in field experiments. Unless the stand of a crop in differently treated plots is uniform and comparable, the effect of various treatments under trial will be obscured. Anything which is likely to assist in obtaining a good stand is equally important for the farmer and for the agricultural experimenter engaged in field experiments.

The stand of a crop depends upon the quantity of seed sown per unit area, its germination capacity and the survival of the seedlings after their emergence from the soil. Seed rate can be varied at will. Germination of seeds when they are viable depends upon (1) the physical nature of soil, (2) its preparation, (3) moisture content and (4) the depth at which seeds are sown. Of all these factors, No. 4 can be measured and controlled with minimum effort, but still very little definite information with regard to the range of depth from which the seeds of common crops can germinate in a particular soil is available. Consequently some work was done in this direction the results of which are presented in this paper.

The depth at which seeds are sown not only affects germination but it may affect the after-growth of the seedlings and consequently influence the yield. Martin [1930] says that potatoes planted half-inch deep gave 16·5 bushels per acre more than deeper plantings. White [1934] found that sweet potatoes covered with one inch sandy soil yielded 2,260 lbs. of primes per acre more than those covered three inches deep. Johnsen and Nielsen [1934] sowed spring cereals at nine depths from zero to twenty cm., spaced at intervals of 2·5 cm., and found that development of seedlings and older plants from seeds planted at depths greater than five cm. was delayed and they showed less vigour and poorer colour. According to Rees [1931] the best depth for sowing sainfoin is 3/4 inch to 1/4 inch.

In order to find out the optimum depths for sowing common crops at Lyallpur their seeds were sown at different depths. This work was primarily undertaken for our own guidance in securing better stand in experimental plots, but it is now felt that this information may be of use to other workers and consequently it is brought to their notice.

II. Method and material

Seeds used in this study were hand-picked. The soil in which these tests were made is loam and is representative of the soils of the district. It contains clay 8·36 per cent, silt 14·63 per cent, fine sand 62·72 per cent and sand 14·3 per cent. It was irrigated in the usual way with canal water and seed-bed was prepared by means of spade. Moisture content of the seed-bed was determined. It generally varied from 11·61 to 12·03 per cent. Seeds were sown by means of sowing tubes devised by the authors [Labh Singh, Nathuram and Nek Alam, 1934] at ten known depths varying from one half to five inches, the interval between consecutive depths being half an inch. Fifty seeds of each crop were sown at each depth in rows 2½ inches apart, the distance from seed to seed in the row being also 2½ inches. There were six such replications in randomised blocks, thus the total number of seeds of each crop sown being 300 at each depth.

Germination tests of each crop were repeated several times in the year but results of only those tests are given here which were made during the main sowing season of each crop. Seeds were sown in the evening. Observations to record germination were commenced twenty-seven hours after sowing and repeated regularly every three hours, *viz.*, 9 P.M., 12 P.M., 3 A.M. and 6 A.M., etc. A seed was considered to have germinated when the seedling had emerged above the ground. At each observation the seedlings that had come above the ground were counted and pulled out so that counting at the next observation may be facilitated.

III. Germination at various depths

Altogether fourteen summer and eight winter crops were dealt with, their local, English and Botanical names are given below :—

Season of growth	Serial No.	Local name	English name	Botanical name
Summer	1	*Rawan* . .	Cowpea . .	*Vigna catjang* Walp.
,,	2	*Mung* . .	Green gram .	*Phaseolus radiatus* L.
,,	3	*Moth* . .	Moth . .	*Phaseolus aconitifolius* L.
,,	4	*Mash* . .	Black gram .	*Phaseolus mungo* L.
,,	5	*Sani* . .	San (hemp). .	*Crotalaria juncea* L.
,,	6	*Gwara* . .	Fieldvetch or Gwara	*Cyamopsis psoralioides* DC.
,,	7	*Makki* . .	Maize. . .	*Zea Mays* L.
,,	8	*Chari* . .	Great millet .	*Andropogon Sorghum* L.
,,	9	*Sudan Ghas* .	Sudan Grass .	*Sorghum sodancuris* L.
	10	*Bajra*	Bulrush millet .	*Pennisetum typhoideun* Rich.

Season of growth	Serial No	Local name	English name	Botanical name
Summer	11	*Swank*	Swank	*Panicum colonum* L.
,	12	*Til*	Sesamum	*Sesamum indicum* D C.
	13	American *kapas*	4-F American	*Gossypium hirsutum* L.
	14	*Desi kapas*	15 Mollisoni	*Gossypium indicum* Lam.
Winter	15	*Chana*	Gram	*Cicer arietinum* L.
,,	16	*Kanak*	Wheat	*Triticum vulgare* Host.
,,	17	*Jaon*	Barley	*Hordeum vulgare* L.
,,	18	*Jai* or *Jawi*	Oats	*Avena sativa* and *A. sterilis* L.
,,	19	*Shalgum*	Turnips	*Brassica rapa* L.
,,	20	*Taramira*	Rocket	*Eruca sativa* L.
,,	21	*Toria*	Indian rape	*Brassica napus* L.
,,	22	*Sarson*	Indian colza	*Brassica campestris* Prain

As already stated germination counts were made after every three hours. The detailed results are given in Appendix I. They are represented below graphically. Fig. 1 relates to fourteen summer crops and Fig. 2 represents eight winter crops :—

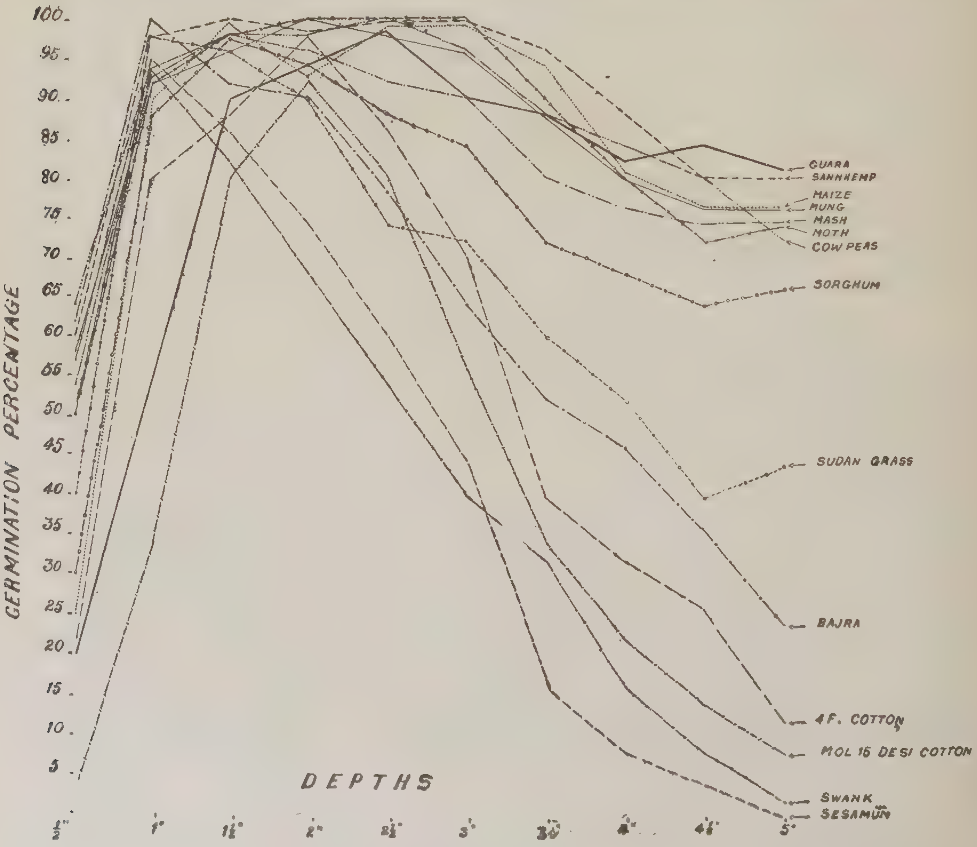

Fig. 1. Showing the germination percentage of fourteen summer crops at different depths.

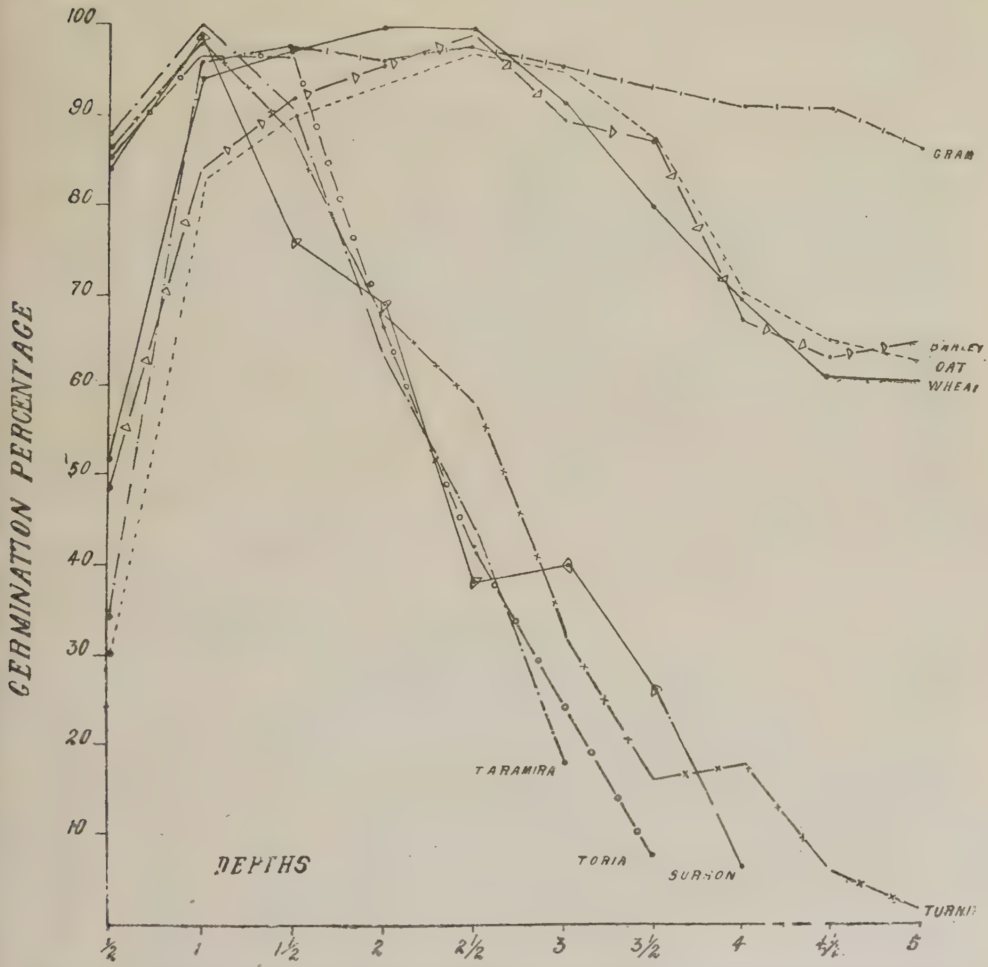

Fig. 2 Showing the germination percentage of eight winter crops at different depths.

The whole data have been examined statistically. The general effect of depths on germination is significant in all the crops. The standard errors of the mean differences have been worked out by analysis of variance and are given in Appendix I referred to above.

It would be seen from Appendix I and Figs. 1 and 2 that each crop has given good germination at more than one depth. For instance, gram has germinated almost equally well at depths 1 to 4½ inches (92-98 per cent). The question now arises as to the optimum depth for sowing this crop. To answer this question the range of depth is fixed by taking germination at higher and lower depths which did not differ significantly from the maximum germination percentage. (Critical difference was taken to be twice the standard error of mean difference). The middle depth of this range where the number of depths is odd, and the first of the two middle-most depths where the number is even, has been taken as the optimum depth of the crop. For instance, eight depths from 1 to 4½ (1, 1½, 2, 2½, 3, 3½, 4, 4½) in the case of gram did not differ significantly from each other. Hence 2½ inches have been taken as optimum depth. Proceeding in this manner the optimum range and optimum depth for each crop has been determined and is given in the following table :—

Serial No.	Crop	Optimum depth in inches	Optimum range of depths in inches	At optimum depth, time of start, finish and period of germination in hours		
				Start	Finish	Period
1	Cowpeas	2	1-3	48	69	21
2	*Mung*	2	1-3	54	75	21
3	*Moth*	2	1-3	54	84	30
4	*Mash*	2	1-3	60	84	24
5	San-hemp	2	1-3½	42	63	21
6	*Gwara*	2½	1½-3½	51	93	42
7	Maize	2½	2-3½	72	108	36
8	*Chari*	1½	1-2½	54	87	33
9	S. Grass	1½	1-2	48	84	36
10	*Bajra*	1½	1-2	42	84	42
11	*Swank*	1	1-1½	60	93	33
12	*Til*	1	1-1½	48	87	39
13	4-F American	2	1½-2½	78	129	51
14	Mollisoni 15 *Desi*	1½	1-2	75	126	51
15	Gram	2½	1-4½	102	123	21
16	Wheat	2	1-3	114	147	33
17	Barley	2½	1½-3½	126	168	42
18	Oats	2½	1½-3½	135	177	42
19	Turnips	1	½-1½	72	99	27
20	*Taramira*	1	½-1½	57	90	33
21	*Toria*	1	½-1½	72	111	39
22	*Sarson*	1	½-1½	72	96	24

In 2nd column of the above table is given the optimum depth and in the 3rd column is given the range of depths between which germinaion was not significantly different from the maximum germination percentage. In order to get good germination, seed should under ordinary conditions be sown at its optimum depth but in those soils which lose moisture rapidly from the top especially during hot and dry weather or when seed-bed is not well prepared it should be sown between the optimum depth and the highest limit of its optimum range. Optimum depth and optimum range would vary according to the character of soil. They would be shallow in stiff and deep in loose soils. Further it would be observed that range in some crops is wide and even an indifferent sowing in their case would give good germination as in case of gram, san-hemp, pulses, etc. They are not so sensitive to depth. The range of some crops such as *swank*, sesamum, cotton, rapes, turnips, etc., is very narrow and to get a good germination of these crops is not always an easy task. They are more sensitive to depth. More care is, therefore, required with regard to depth while sowing these crops.

IV. TIME TAKEN FOR THE START AND COMPLETION OF GERMINATION BY DIFFERENT CROPS

As stated in the beginning observations were started twenty-seven hours after sowing and were repeated after every three hours. It was observed that some crops started to germinate earlier than others although they were sown at the same time and other conditions were kept as uniform as possible. The time when germination was completed was also noted in each case. The difference in the time of start and the time of completion of germination is the period during which a crop germinated. This interval is called the period of germination. Detailed data in this connection are given in Appendix II at the end.

A reference to Appendix II would indicate that in general the shallower the depth the quicker is the start of germination. Maize, gram and *gwara* took, however, longer to start germination at ½ inch than at one inch. Other main features of Appendix II are :—

(1) Almost all seeds at half an inch took longer time to complete germination than at one inch, probably because soil moisture is less in the first half inch of soil than in the second half inch (at one inch depth).

(2) On the whole, period of germination is smaller at depths lower than the optimum depth within the optimum range.

V. GERMINATION AT VARIOUS HOURS OF THE DAY

As already stated germination counts were taken after every three hours throughout the day and night, *viz.*, 3 A.M., 6 A.M., 9 A.M., etc. In this way seeds that germinated between 3 A.M. and 6 A.M. were recorded at 6 A.M. and those germinated between 6 A.M. and 9 A.M. were noted at 9 A.M. and so on. It was observed that the rate of germination at all depths and more or less for all crops is rapid during certain hours whereas at others it was very slow. Maximum germination took place between 3 A.M. and 6 A.M. It was next followed by the interval between midnight and 3 A.M. During noon hours germination proceeded very slowly. Least germination took place during the hours from 6 to 9 P.M. The rate of germination during the different hours of the day for all crops at their optimum depth is given in Appendix III.

The average germination percentage of all crops that took place at different hours of the day is given below :—

Period of the day (hours) . .	0-3	3-6	6-9	9-12	12-15	15-18	18-21	21-24
Germination percentage . .	18·1	36·4	16·7	5·4	4·2	7·1	2·4	7·4

The figures of germination percentage given in the 2nd row above are the result of sowing 6,600 seeds of the twenty-two crops under observation.

In crops as pulses and san-hemp germination, once it has started, is finished more or less within first twenty-four hours. In cotton it takes more than forty-eight hours. In the case of crops which take more than twenty-four hours for the completion of germination, the rate of germination during the various hours of the day during the second twenty-four hours was in the same order as in the first twenty-four hours.

In all the tests the data relating to which have so far been discussed seeds were sown at 6 P.M. In order to see whether seeds sown at different hours of the day would behave in a similar manner, Mollisoni 15 Desi and 4-F American cottons were sown at their optimum depths on the 24th May, 1933, at 6 P.M., 7 A.M. (next morning) and 12 noon. The land for all these sowings was prepared at one and the same time. Observations were recorded after every hour in this case. The mean maximum and minimum temperature and relative humidity during the days of the experiment were 108·1°F. and 76·1°F. and 36·8 per cent respectively. The detailed data recorded after every one hour are given in Appendix IV. To make it comparable with the previous data, recorded after every three hours, data recorded after every one hour was grouped into periods of three hours, *viz.*, 3-6, 6-9, etc., etc., and the results of the grouping are given below :—

Variety	Time of sowing	No. of hours after sowing when germination started	Hours of the day when germination started	Percentage germination during the hours from								
				0 to 3	3 to 6	6 to 9	9 to 12	12 to 15	15 to 18	18 to 21	21 to 24	Total
Mollisoni 15 *Desi*	6 P.M. . .	60	6 A.M. .	10	16	34	14	2	2	...	12	90
	7 A.M. (next day)	66	1 A.M. .	18	34	30	8	...	...	...	4	94
	12 NOON . .	61	1 A.M. .	26	38	24	...	...	...	...	4	92
4-F American .	6 P.M. . .	62	8 A.M. .	16	24	34	4	...	...	2	14	94
	7 A.M. (next day)	67	2 A.M. .	18	32	34	6	...	...	...	8	98
	12 NOON . .	61	1 A.M. .	18	38	32	4	...	...	4	4	100

These figures in general confirm the previous observations, *viz.*, greater percentage of seeds germinated during the cool hours after mid-night irrespective of the time of sowing. After 9 A.M. germination declines steadily. There is very little germination in the afternoon and evening.

Another interesting point which would be observed from the foregoing table is that *desi* cotton sown at 6 P.M. started to germinate sixty hours after sowing whereas cotton sown at 7 A.M. took sixty-six hours and cotton sown at 12 noon sixty-one hours. 4-F American has also behaved in the same manner. The question arises what is this difference due to ? Apart from other things seed would germinate after it has been in the soil for a certain period and if this period ends when the hours to follow are favourable for germination it begins to germinate, but if at the end of this period the following hours are not favourable for germination, it seems as if the crop waits for favourable time to come. *Desi* cotton sown at 6 P.M. completed sixty hours after sowing at 6 A.M., a time which is favourable for germination. So it began to germinate. On the other hand, cotton sown at 7 A.M., completed its sixty hours at 7 P.M. when time was not favourable for germination. It had, therefore, commenced to germinate at 1 A.M. and thus took sixty-six hours to start germination. Noon sowing commenced germinating after sixty-one hours at 1 A.M., when time was favourable for germination. 4-F American cotton has also behaved in the same manner.

Should land be ready in the afternoon, at present the practice is to sow summer crops in the evening even if men and bullocks have to be over-strained because it is supposed that evening sowings give better germination than sowings made next morning. The experiments described above, however, clearly show that evening sowings have no special advantage over sowings done next morning. After the preparation of land the question whether summer crops should be sown on the same evening or next morning should solely be decided according to the facilities available and not by the necessity of sowing at a particular hour of the day.

SUMMARY

(1) A good stand of a crop is important for the practical farmer as well as the agricultural experimenter. The depth at which seed is sown not only affects the germination percentage of seed and the survival of young seedlings but also the ultimate stand of a crop. Seeds of twenty-two common crops were sown at the depths from half to five inches, interval between the successive depths being half inch, *viz.*, ½, 1, 1½, etc., and germination counts were recorded after every three hours.

(2) Optimum depth in loam soil for turnips, *taramira*, *toria*, *sarson*, sesamum and *swank* is one inch ; for sorghum, Sudan grass, *bajra*, and *desi* cotton 1½ inches for cowpeas, *mung*, *moth*, *mash*, sann-hemp, 4-F American cotton and wheat two inches and for *gwara*, maize, gram, barley and oats 2½ inches.

(3) Irrespective of the depth and the time at which seeds were sown the rate of germination was very rapid from midnight to 9 A.M., while it was very low from noon to midnight.

(4) Practical farmers in the Punjab believe that sowing of summer crops in the evening gives better germination than sowing next morning. The results obtained in this test do not confirm this belief.

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APPENDIX I

Percentage of seeds that germinated from different depths

DEPTHS IN INCHES

No.	Crop †	½″	1″	1½″	2″	2½″	3″	3½″	4″	4½″	5″	S. E. of the M. D.*
1	Cowpeas	64	94	98	98	100	96	88	84	80	72	3·4
2	*Mung*	58	92	96	100	98	96	88	80	76	76	3·6
3	*Moth*	50	92	98	100	100	100	90	80	72	74	4·1
4	*Mash*	54	92	98	96	92	90	80	76	74	74	3·9
5	Sann-hemp . . .	60	98	100	98	100	100	96	88	80	80	2·4
6	*Gwara*	20	56	90	94	98	90	88	82	84	80	4·2
7	Maize	4	34	80	92	100	100	94	80	76	76	4·2
8	Sorghum . . .	30	88	98	94	88	84	72	68	64	66	4·6
9	Sudan Grass . .	40	98	96	90	74	72	60	52	40	44	3·9
10	*Bajra*	60	100	92	90	78	64	52	46	36	24	4·1
11	*Swank* . . .	52	92	82	68	54	40	32	16	8	2	5·4
12	Sesamum . . .	58	94	86	74	60	44	16	8	4	...	4·7
13	4-F. American cotton .	20	80	88	98	86	70	40	32	26	12	7·1
14	Mollisoni 15 Desi . .	22	90	100	92	80	56	34	22	14	8	6·8
15	Gram	34	96	98	96	98	96	94	92	92	88	2·7
16	Wheat . . .	52	94	98	100	100	92	80	70	62	62	4·2
17	Barley . . .	48	84	92	96	100	90	88	68	64	66	5·6
18	Oats	30	84	90	94	98	96	88	70	66	64	4·9
19	Turnips . . .	86	98	88	68	58	32	16	18	6	2	6·3
20	Taramira . . .	88	100	90	64	44	18	...	...	...	...	6·9
21	*Toria*	86	96	96	66	42	24	8	...	...	...	4·9
22	*Sarson* . . .	84	98	86	68	38	40	26	6	...	...	7·4

* This has been calculated on the basis of the S. D. per plot calculated by Fisher's analysis of variance.

† Botanical names of crops are given in Table I.

APPENDIX

The number of hours after which germination started and

No	Crops	DEPTH ½			1			1½			2		
		S	F	P	S	F	P	S	F	P	S	F	P
1	Cowpeas . .	39	84	45	39	60	21	48	66	18	48	69	21
2	*Mung* . . .	42	87	45	48	66	18	54	72	18	54	75	21
3	*Moth* . . .	33	69	36	42	63	21	54	84	30	54	84	30
4	*Mash* . . .	39	87	48	39	69	30	54	81	27	60	84	24
5	Sann-hemp . .	30	63	33	33	48	15	39	60	21	42	63	21
6	*Gwara* . . .	54	99	45	48	87	39	48	87	39	48	90	42
7	Maize . . .	87	114	45	69	114	45	72	105	33	72	108	36
8	Sorghum . .	45	108	63	54	87	33	54	87	33	60	105	45
9	Sudan Grass .	45	108	63	45	81	36	48	84	36	48	90	42
10	*Bajra* . . .	36	84	48	39	84	45	42	84	42	48	87	39
11	Swank . .	60	132	72	60	93	33	60	108	48	60	108	48
12	Sesamum . .	54	72	18	48	87	39	57	108	51	57	135	78
13	4-F American cotton.	75	132	57	78	132	54	78	132	54	78	129	51
14	Mollisoni 15 Desi .	69	129	60	72	126	54	75	126	51	75	129	54
15	Gram . . .	108	171	63	96	117	21	99	120	21	99	120	21
16	Wheat . .	102	141	39	105	135	30	105	138	33	114	147	33
17	Barley . .	108	183	75	120	168	48	120	168	48	120	168	48
18	Oats . . .	126	186	60	129	186	57	132	180	48	132	186	54
19	Turnips . .	60	102	42	72	99	27	93	135	42	96	147	51
20	*Taramira* . .	54	105	51	57	90	33	63	111	48	81	132	51
21	*Toria* . . .	60	108	48	72	111	39	84	120	39	84	156	72
22	*Sarson* . .	63	111	39	72	96	24	72	111	39	84	132	48

S—Denotes the No. of hours after sowing when germination started.

F—Denotes the No. of hours after sowing when germination was completed.

P—Denotes the No. of hours during which germination took place.

II

finished and period of germination at different depths

IN INCHES

2½			3			3½			4			4½			5		
S	F	P	S	F	P	S	F	P	S	F	P	S	F	P	S	F	P
51	90	39	54	102	48	57	111	54	57	111	54	63	111	48	63	114	51
54	90	36	57	90	33	60	99	39	60	114	54	63	126	63	66	126	60
54	96	42	57	102	45	57	108	51	66	126	60	69	132	63	69	138	69
60	87	27	66	93	27	69	108	39	69	111	42	69	138	69	78	138	60
45	75	30	45	87	42	48	96	48	48	96	48	51	120	69	51	126	75
51	93	42	54	114	60	60	120	60	63	123	60	63	132	69	78	150	72
72	108	36	75	114	39	78	114	36	78	114	36	78	117	39	78	117	39
60	114	45	60	114	54	60	114	54	60	114	54	63	114	51	63	120	57
54	99	54	57	114	57	57	126	69	60	135	75	60	135	75	69	144	75
48	90	42	48	108	60	54	114	60	63	132	69	63	144	81	81	153	72
60	111	51	66	114	48	72	114	42	72	114	42	84	114	30	84	114	30
72	132	60	81	138	57	84	138	54	84	156	72	120	156	36	...	...	...
81	156	75	81	156	75	81	165	84	84	204	120	90	213	123	132	249	117
78	153	75	81	159	78	81	162	81	87	207	120	87	222	135	99	222	123
102	123	21	108	138	30	114	159	45	114	165	51	120	168	48	126	171	45
114	144	30	117	147	30	123	156	33	129	177	48	132	183	51	141	189	48
126	168	42	126	171	45	132	177	45	144	192	48	150	201	51	150	204	54
135	177	42	135	183	48	150	201	51	156	207	51	159	207	48	162	207	45
101	168	67	144	171	27	144	171	27	150	180	30	153	183	30	168	192	24
84	144	60	87	156	69	...	...	...	...	...	...	...	...	...	...	...	...
84	159	75	102	159	57	105	159	54	...	...	...	...	...	...	...	...	...
87	138	51	90	156	66	108	156	48	132	156	24	...	...	...	...	...	...

APPENDIX III

Percentage of seeds germinated during the different hours of the day from optimum depth for each crop

No.	Crop	Opt-mum depth in inches	0-3	3-6	6-9	9-12	12-15	15-18	18-21	21-24	Total
1	Cowpeas . .	2	24	24	12	2	2	14	..	20	98
2	*Mung* . .	2	34	30	8	..	6	2	4	16	100
3	*Moth* . . .	2	24	38	20	8	2	2	2	4	100
4	*Mash* . . .	2	10	22	30	6	12	10	4	2	96
5	Sann-hemp . .	2	18	26	4	4	4	2	14	26	98
6	*Gwara* . . .	2½	28	26	16	6	..	6	6	10	98
7	Maize . . .	2½	22	34	18	8	6	..	2	10	100
8	Sorghum . .	1½	24	44	20	4	..	2	..	4	98
9	Sudan Grass . .	1½	8	38	18	2	8	16	6	2	98
10	*Bajra* . . .	1½	4	36	8	2	6	22	2	12	92
11	*Swank* . . .	1	2	52	8	8	4	16	..	2	92
12	Sesamum . .	1	8	42	26	10	4	2	2	..	94
13	4-F American cotton	2	22	32	24	6	4	4	..	6	98
14	Mollisoni 15 *desi* .	1½	26	34	22	4	2	2	2	8	100

15	Gram . . .	2½	8	38	8	8	6	14	4	12	98
16	Wheat . . .	2	12	26	20	8	6	16	4	8	100
17	Barley . . .	2½	34	28	14	6	10	..	..	8	100
18	Oats . . .	2½	20	48	20	8	..	..	..	2	98
19	Turnips . .	1	18	52	18	2	4	4	..	..	98
20	*Taramira* . .	1	14	38	26	10	4	8	..	..	100
21	*Toria* . . .	1	28	36	16	2	2	4	..	8	96
22	*Sarson* . . .	1	10	56	12	4	..	10	2	4	98
	Average . .		18·1	36·4	16·7	5·4	4·2	7·1	2·4	7·4	97·7

APPENDIX

Germination percentage of cotton (Desi and American) in

Variety	Time of sowing	No. of hrs. after which germination started	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Mollisoni 15 *desi*	6 P.M. .	60	...	...	...	...	...	2	4	4	8	8	2	...	...	2	...	...	...
	7 A.M. .	66	6	2	6	4	10	6	14	8	6	6	2	...	...	...	...	...	...
	12 NOON	61	2	6	8	14	8	8	10	8	2	...	...	...	...	...	...	...	...
4-F American	6 P.M. .	61	...	...	...	...	...	...	...	2	6	2	...	...	...	...	...	...	...
	7 A.M. .	67	...	4	8	4	8	6	10	8	14	4	2	...	...	...	...	...	...
	12 NOON	61	4	4	6	4	14	16	8	10	12	4	...	...	...	...	...	...	...

IV

each hour of the day when sown 2 inches deep at different hours of the day

18	19	20	21	22	23	24	1	2	3	4	5	6	7	8	9	10	11	12	After-wards upto 144 hours	Total
2	...	...	...	2	8	2	2	4	4	2	2	6	8	4	6	4	...	...	4	92
...	...	...	...	...	2	2	2	...	2	4	4	4	2	...	...	...	...	...	2	94
...	...	...	...	...	2	2	4	2	4	...	2	2	...	2	...	...	...	...	6	92
...	...	2	...	4	4	6	6	4	6	4	6	14	...	12	26	2	...	...	6	94
...	...	...	...	2	4	2	2	4	...	2	4	2	...	2	...	...	...	...	6	98
...	2	2	...	...	2	2	...	2	2	...	...	4	...	...	2	...	...	...	...	100

A PHOMA DISEASE OF ASPARAGUS

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(Received for publication on 19th February 1936)

(With Plate XLIII)

Asparagus is extensively grown in some parts of Europe and America from where canned asparagus are exported. In India its cultivation is limited to certain parts of the country and in addition to its use as a vegetable, the berries are often employed medicinally by the *hakims*.

SYMPTOMS

In 1935 asparagus plants at Pusa were found to be affected by a fungus disease. The first symptom is the discoloration of the tissue of the stem into light brownish areas which become dark brown as the disease advances. The affected parts become shrivelled but do not collapse (Plate XLIII, A). The tissues at the centre of the spots become ashy white and later show small dark bodies. These are seen on microscopic examination to be the pycnidia of a fungus. The pycnidia break through the epidermis which cracks longitudinally. The growth of lateral branches is frequently checked (Plate XLIII, B). The disease appears on all parts of the plants except the berries but is principally confined to the main stem. In advanced cases the needles become yellow, and the plants may become completely or partially defoliated, and finally wither. The pith of the affected stem becomes ashy white and spongy, owing to the invasion of the fungus hyphae.

PATHOGENICITY

Cultures of the fungus were obtained by planting sterilised bits of affected tissues on Quaker-oats agar slants and from these pure cultures were re-isolated.

Several successful inoculation experiments were made at Pusa during the rains and autumn on unwounded and wounded asparagus stems and pathogenicity of the fungus was thus proved. The method employed was to place fragments of culture or of naturally infected tissue in contact with the healthy stems—either with or without wounding—and to keep moist by binding round with damp cotton wool. As might be expected infection took place more rapidly on wounded than on unwounded plants. The influence of climatic conditions on the incidence of the disease was indicated by observation that both natural and artificial infection took place more readily during the monsoon than during the following months. The fungus was re-isolated from the inoculated parts and compared with the parent culture and was found to be identical.

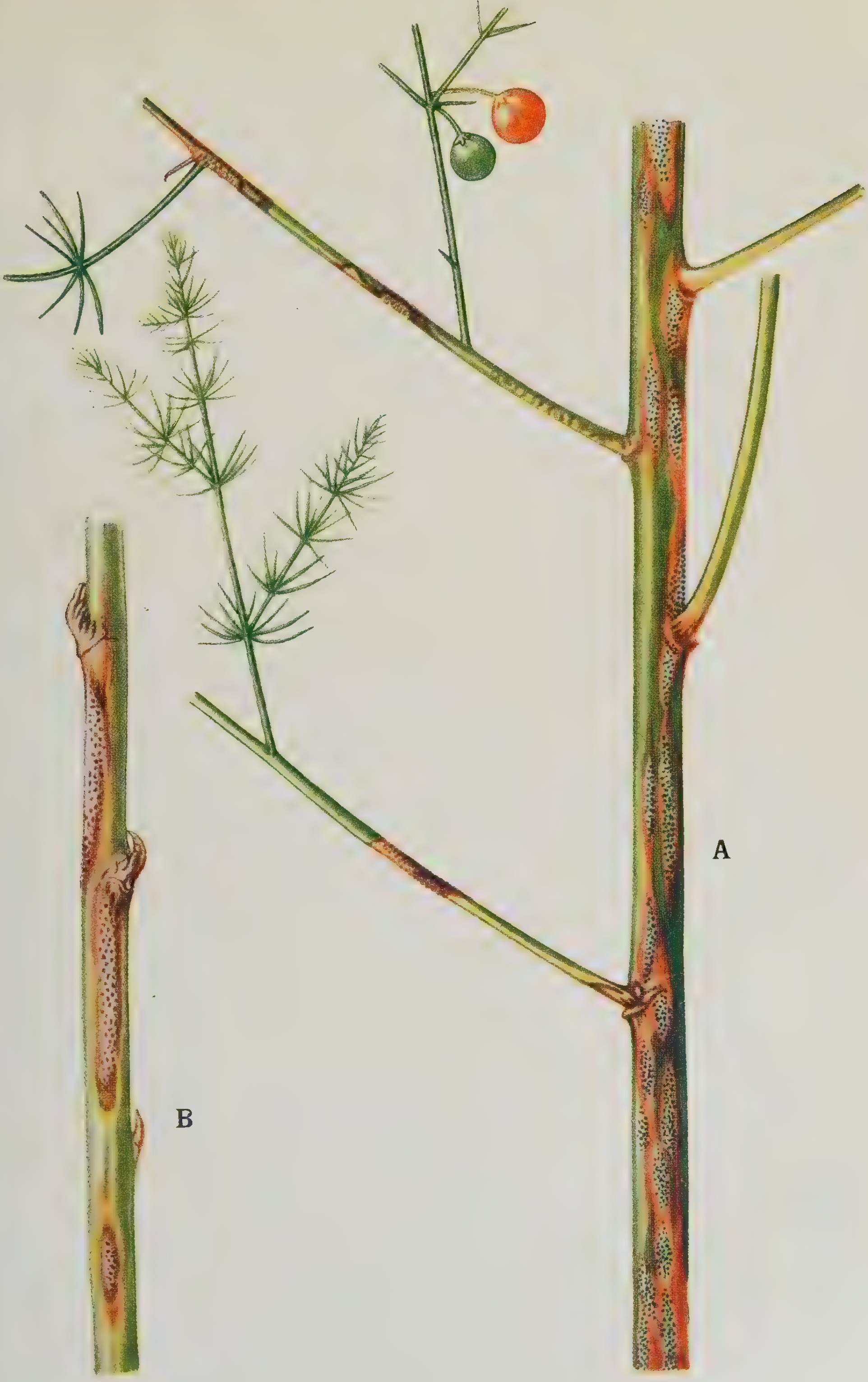

FIG. (A) Asparagus stem showing the attack of the fungus $(\times 1\frac{1}{5})$

FIG. (B) Young asparagus stem showing withered lateral buds $(\times 1\frac{1}{5})$

MORPHOLOGY OF THE FUNGUS IN CULTURE

On rich nutrient media like Quaker-oats agar the fungus grows profusely Growth is at first confined to the substratum, and when the fungus reaches the edge of the slant aerial mycelium is formed. The mycelium is at first hyaline and then turns light brown, and the hyphae vary in breadth. As the culture grows old vesicles are formed in the hyphae. Light brown pycnidia are formed at the margin of the agar slant, but fail to mature. On sterilised fragments of asparagus stems, however, pycnidia with numerous pycnospores are readily formed. On Dox's agar, Brown's agar, and Hopkin's agar the growth is poor, and pycnidia are rarely formed.

MICROSCOPIC CHARACTERS

Sections were made of the infected stems of asparagus and examined under a microscope. The mycelium is composed of septate hyaline threads which ramify in all parts of the stem. It is both intra and inter-cellular. Comparatively little mycelium is found in the sclerenchymatous tissues, but in the bundles and the pith it is found abundantly. The pycnidia are usually borne under the epidermis and when they are mature they break through it. The conidia are oblong, rod or spindle-shaped, hyaline and one-celled. They measure $5{\cdot}1$–$10{\cdot}2 \times 1{\cdot}7$–$3{\cdot}4\mu$. The conidiophores are not distinctly visible.

SYSTEMATIC POSITION

The fungus was first described by Fuckel [1869] as the spermogonial form of *Diaporthe asparagi* Fuckel, and Saccardo [1884] who named it *Phoma asparagi* Sacc. was also inclined to regard it as a pycnidial stage of *Diaporthe.* Bubak [1906] stated that in *P. asparagi* he found A and B forms of spores and all variations between them and re-named the species *Phomopsis asparagi* (Sacc.) Bubak. He described the A spores as oblong or spindle-shaped and gave the extreme variations of the size as $5{\cdot}5$–$9 \times 2{\cdot}2$–5μ. The range of 200 spores of the Pusa fungus is $5{\cdot}1$–$10{\cdot}2 \times 1{\cdot}7$–$3{\cdot}4\mu$ which agrees with the A form of spores of Bubak. As the B form of spores are not found in the Indian material it cannot be *Phomopsis.* It may also be stated that Diedicke [1912] who had made a special study of the genus *Phomopsis* did not accept *Phoma asparagi* as a *Phomopsis* but listed it under *Phoma.* Grove [1935] lists the species both as *Phoma* or *Phomopsis.* Teng [1932] has recorded *Phoma asparagi* from China. There are three distinct species of *Phoma* known on asparagus. The present species agrees with *Phoma media* Ell. et Ev. in the size of spores but differs from it in not having distinct conidiophores. It also differs from *Phoma lanceolata* (C. et E.) Sacc. in having much smaller spores. It closely agrees with *Phoma asparagi* Sacc. in the shape and size of spores, structure of pycnidia, and therefore it is considered to be *Phoma asparagi* Sacc. This appears to be the first record of the fungus in India.

ACKNOWLEDGMENT

Thanks are due to Mr. E. W. Mason of the Imperial Mycological Institute, for the help rendered in the identification of the fungus, and for references to the literature.

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GUMMOSIS OF CITRUS IN BOMBAY

BY

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(With Plates XLIV-XLVI and two text-figures)

INTRODUCTION

Gummosis, one of the commonest and most destructive maladies of citrus, has an extensive geographical distribution. In the Bombay Presidency it was first recorded on the pomelo, **Citrus grandis* Osbeck, in 1910, although it is probable that the disease had existed in the citrus orchards for a long time. The disease, however, did not attract much attention until 1923 when serious outbreaks were reported on sweet orange locally known as *mosambi*, a horticultural variety of *Citrus sinensis* Osbeck, from important centres of citrus cultivation. In a few years the disease has been widely distributed, and there is now hardly a locality in the Bombay-Deccan free from it.

Fawcett [1923] published the results of his experiments in 1923, but since he came to the conclusion that pathological effects associated with gum formation were produced by a number of independent organisms, it was considered necessary to undertake an investigation into the nature of the disease as it occurs in Bombay, the conditions influencing its development, and the method of prevention and treatment. Unfortunately, there have been many interruptions in the progress of the work, and it has thus not been possible to publish these results at an earlier date. This paper embodies the results obtained during the period from 1925 to 1932, inclusive.

NOTE—The writers acknowledge the assistance rendered by Mr. M. K. Desai, formerly Ag. Assistant Mycologist, in field experiments.

* The names of species and varieties of Citrus used in this paper are according to W. T. Swingle (" Citrus " in Bailey's Standard Cyclopaedia of Horticulture, Vol. 2, pp. 270-785, New York, 1914) and G. S. Cheema and S. S. Bhat (" A Study of the Citrus Varieties of the Bombay Presidency " in *Current Science*, Vol. 2, pp. 298-304 [1934.)

A valuable review of literature dealing with gummosis and brown rot of citrus has been published by Fawcett [1923, 1 ; 1923, 2.]

SYMPTOMS

In Bombay gummosis produces typical symptoms in *mosambi* and affects the trunk. In the field it is characterised by the copious exubation of a resinous gummy substance and the cracking of the bark for considerable distances upwards from the bud union (Plate XLIV, figs.1 and 2) ; in an advanced case of attack there may be extensive shedding of the bark exposing the wood below (Plate XLIV, fig. 3). This is followed by the yellowing and drooping of leaves, and the affected trees look as if suffering from the effects of a drought (Plate XLIV, fig. 4).

In the early stage of the disease, if the bark in the invaded region is scraped with a knife, the inner surface shows brown discoloration which gradually fades at the margins of the invaded zone into the normal colour of the sound tissue (Plate XLV, fig. 1). It is beyond the invaded region that the fungus is most actively growing. The bark remains firm at first, but ultimately shrinks and cracks longitudinally.

In time the invaded region extends considerable distances upwards from the bud union but relatively smaller distances sideways. Invariably there is a rapid upward extension from the bud union, and the bark may also crack longitudinally 50 to 60 cm. or more in length. The most serious aspect of the disease is in the lateral extension of the invaded region, and when more than two-thirds of a trunk is girdled, treatment becomes difficult and the tree eventually dies (Plate XLIV, figs. 3 and 4).

In the area surrounding an advanced region, a watery substance is produced underneath the bark, and as the gum accumulates in large quantities near the cambium, the bark is separated from the wood below, thus forming gum pockets. The pressure ultimately is so great that the bark cracks longitudinally and masses of watery gum come to the surface where they soon dry by loss of water. There is often such a copious exudation of gum that masses of it run down the trunk and accumulate at the base in the dry season.

In badly affected trees the dead bark dries and sloughs off. The wood thus exposed, if unprotected, is attacked by the wood-rotting fungi and decays.

In wet or damp weather the fruit is also attacked. During the rains the fungus produces the active motile spores or zoospores, which are the principal agents in the spread of infection. These zoospores are splashed from the soil to fruits on branches almost touching the ground, and germinate by hyphae which enter the rind or underlying tissue. After a few days an oily spot appears on the surface (Plate XLV, fig. 2 (*a*)), which rapidly enlarges in extent until the whole fruit is involved. The affected fruits fall to the ground, and the fungus soon comes out on the surface as small white patches of mycelium.

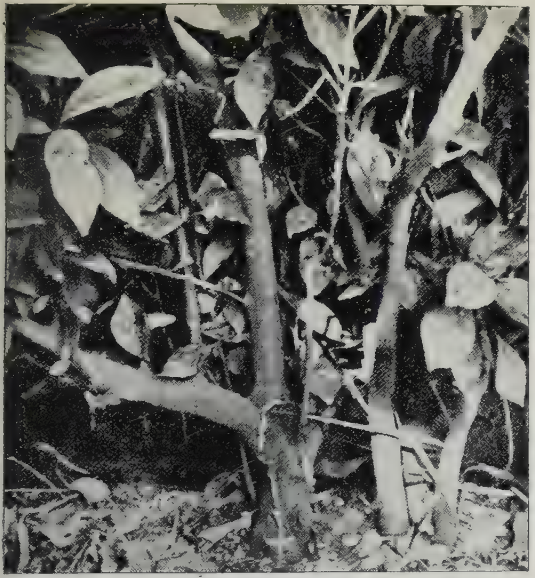


FIG. 1. A *mosambi* tree affected with gummosis showing cracking of bark.

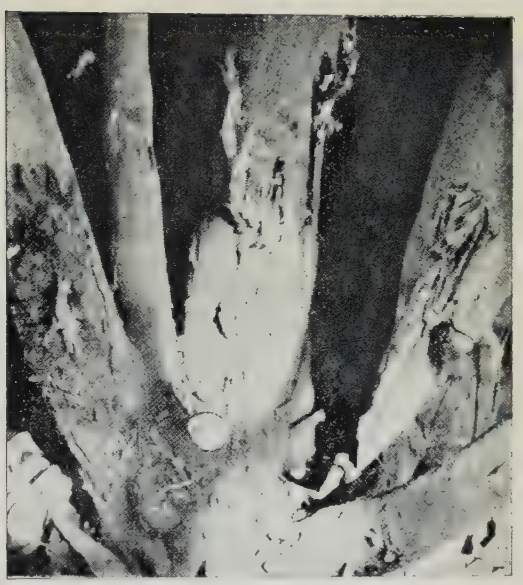


FIG. 2. Same as Fig. 1 and accompanied by copious exudation of gum.

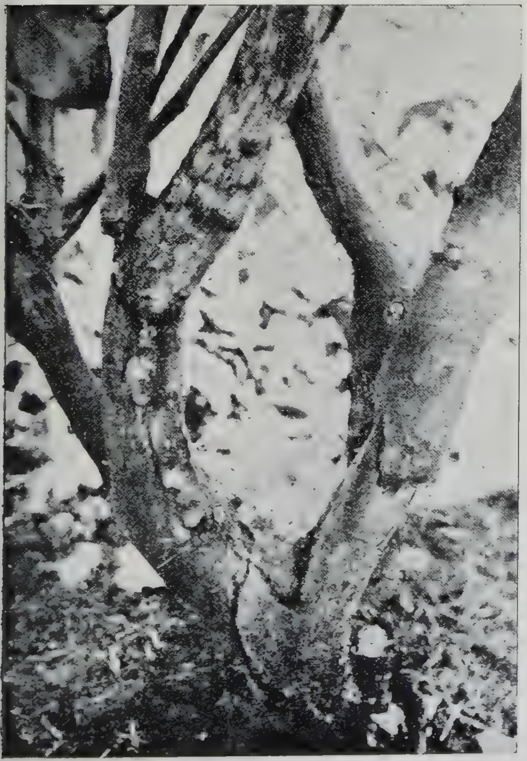


FIG. 3. An advanced case of gummosis showing extensive shedding of bark, resulting in the death of the tree.

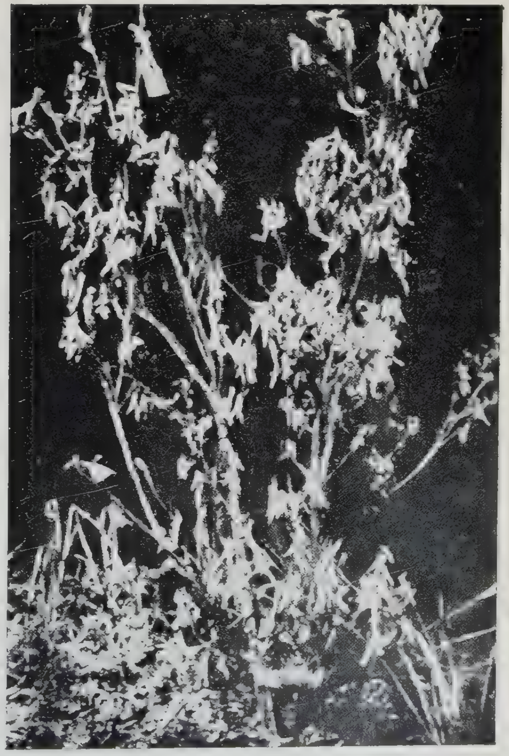


FIG. 4. A severely affected tree showing drooping of leaves. The tree ultimately succumbed to the disease.

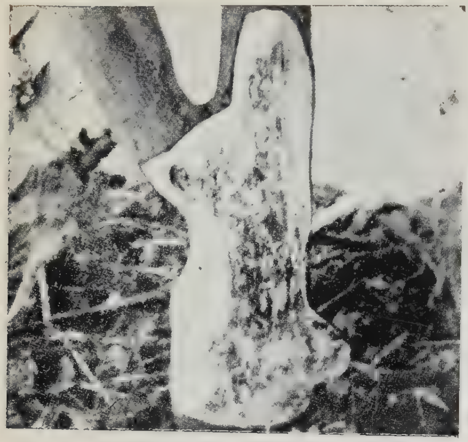


FIG. 1. An affected tree in which diseased bark was removed with a knife. Note an area of brown discoloration in the centre surrounded by healthy tissue.

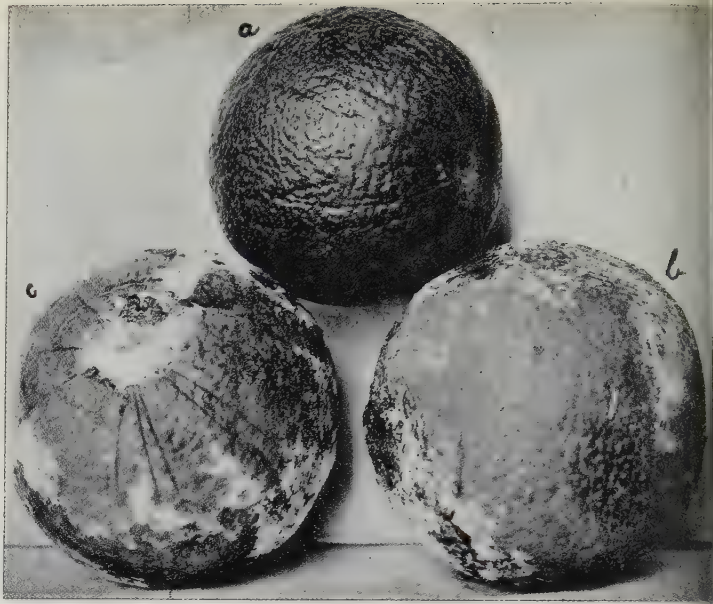
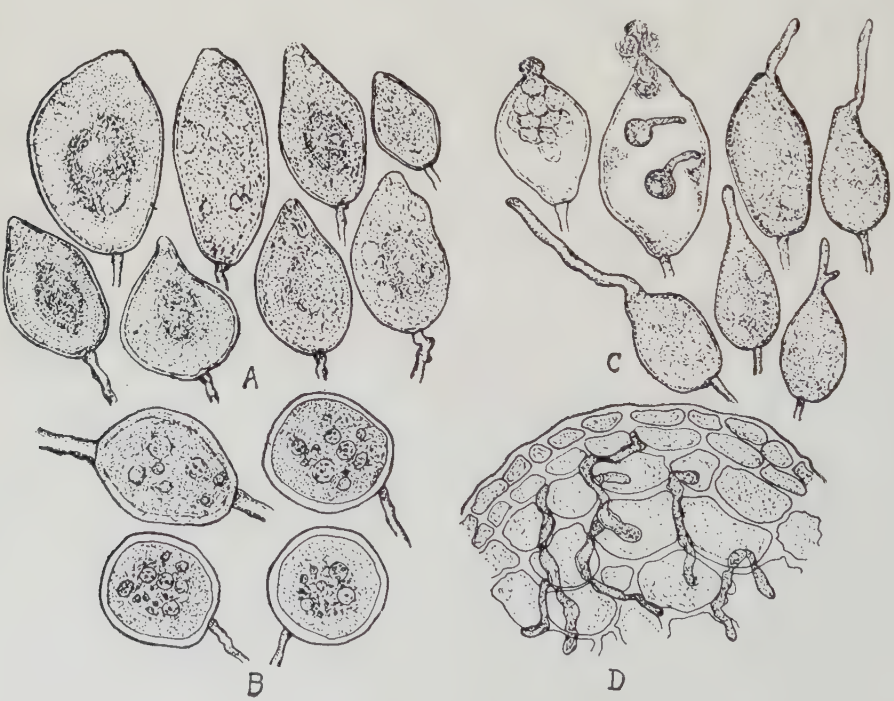


FIG. 2. (*a*) A *mosambi* fruit showing an oily spot due to infecti
(*b*) and (*c*) Note mycelial growth on the surface of affect
fruits after they have been kept under a bell-jar for
few days.



Phytophthora palmivora.

FIG. 3. *A*. Mature sporangia of common shapes and sizes each showing a prominent papilla of dehiscence. *B*. Intercalary and terminal chlamydospores. *C*. Sporangia showing characteristic germination by formation of zoospores and by hyphae. *D*. Section through a portion of affected rind showing intercellular and intracellular mycelium.

As the disease progresses, the colour of the affected fruit changes to dull brown. Under field conditions, the fungus has never been observed to produce superficial growth on diseased fruits still hanging on the trees, but growth appears after an affected fruit has been kept under a bell-jar for a few days (Plate XLV, fig. 2 (*b*) and 2 (*c*)). The odour of the affected fruit is very characteristic and quite pleasant, but becomes fetid through the invasion of the fruit by secondary decay organisms. The affected fruit remains firm and intact until secondary organisms have reduced it to a mass of putrefying tissue.

In the Bombay Presidency brown rot is very common on *mosambi*, but has also been noticed on *santra* fruits (*Citrus nobilis* Lour. var. *deliciosa* Swingle). It resembles in all respects the California brown rot of lemon, *Citrus limonia* Osbeck, caused by *Phytophthora citrophthora* (Sm. & Sm.) Leonian [Smith, 1907].

DISTRIBUTION AND ECONOMIC IMPORTANCE

There are no published records of the occurrence of citrus gummosis in India, which is widely distributed in the Bombay-Deccan. It occurs in a serious form in the Deccan and, although it is very destructive in East Khandesh, its absence in the Central Provinces can only be explained on the basis of unfavourable weather conditions, since Mr. J. F. Dastur in a personal communication reports that the varieties of citrus grown in the Central Provinces either for stock or for fruit are the same as those commonly found in Bombay. The disease has not been reported from Gujarat and the Karnatak. Narasimhan [1927], however, has recorded *Phytophthora arecae* (Coleman) Pethybridge on fruits of *Citrus medica* and *Citrus limonia* in Mysore, but he has not reported whether this fungus can also cause gummosis in citrus trees.

The rapid extension of gummosis in the cortex occurs mainly during wet weather, the injury commencing as the monsoon breaks and continuing till the advent of hot weather. It is particularly severe in months of heavy rainfall and during the cold season when late rains are received in heavy quantities. Under such conditions infection spreads rapidly in the orchards, and also the brown rot of fruits becomes very prevalent.

Gummosis attacks citrus trees of all ages, but is often very destructive in young plantations. On the Deccan Canals the disease appears in more or less serious proportions and the damage may be as high as twenty to twenty-five per cent in many orchards. Table I brings out the extent of damage in *mosambi* orchards at a few centres in the Deccan. At Utran in East Khandesh, gummosis has caused very heavy losses in the largest citrus orchard in the Bombay Presidency. The disease is thus a serious menace to the economic growing of citrus in Bombay, and even the death of a few valuable trees in an orchard may mean a serious loss to a grower.

TABLE I

Showing the extent of gummosis in mosambi *trees in the Deccan (survey of 1924-25)*

Place	No. of trees examined	No. of trees infected	Per cent infection
Baramati	500	65	12
Poona	336	75	21
Junnar	800	201	25
Poona	1,121	110	9
Rahata	1,200	256	21
Poona	1,800	108	6
Loni	1,000	60	6

PATHOGENICITY

Isolation.—In the early stages of this work the fungus was isolated from the diseased bark just beyond the invaded region, since in this region the fungus is making the most active growth. It was also obtained from naturally infected *mosambi* fruits. More than 160 isolations have been made during the course of this work, the majority of the isolations being from the diseased fruits. In all these cases a species of *Phytophthora* was obtained in pure culture. The fungus can also be easily brought into culture from *mosambi* fruits kept in contact with wet, infected soil collected from the base of heavily diseased trees. The fungus enters the fruit from the infected soil and may easily be isolated by transferring sterilised bits of diseased rind to tubes of oat-meal agar.

The material for isolation was carefully selected from young lesions on the stem and from infected fruits which were still firm and emitting a characteristic pleasant odour, as it had been the experience of one of us [Kamat, 1927] that, when isolations were made from older lesions on the stem and from fruits which had become soft and fetid, secondary organisms like bacteria and *Fusarium* were sometimes present in the culture besides the usual *Phytophthora*. The material from which isolations were made was first disinfected in a one in 1,000 solution of mercuric chloride for three minutes and washed in several changes of sterile, distilled water. It was then placed in tubes of oat-meal agar and incubated at room temperature of 25° C.

Inoculations.—Several young *mosambi* plants in pot-cultures were inoculated with two-week-old cultures of the *Phytophthora* on citrus grown on oat-meal agar. A T-shaped slit was made with a sharp knife in the bark at the bud union, and bits of mycelium from young cultures were applied to the cut surface under aseptic conditions. A similar slit was made on the other side of the union, but no mycelium was applied in this case. Cotton wool was wrapped over these slits and was kept

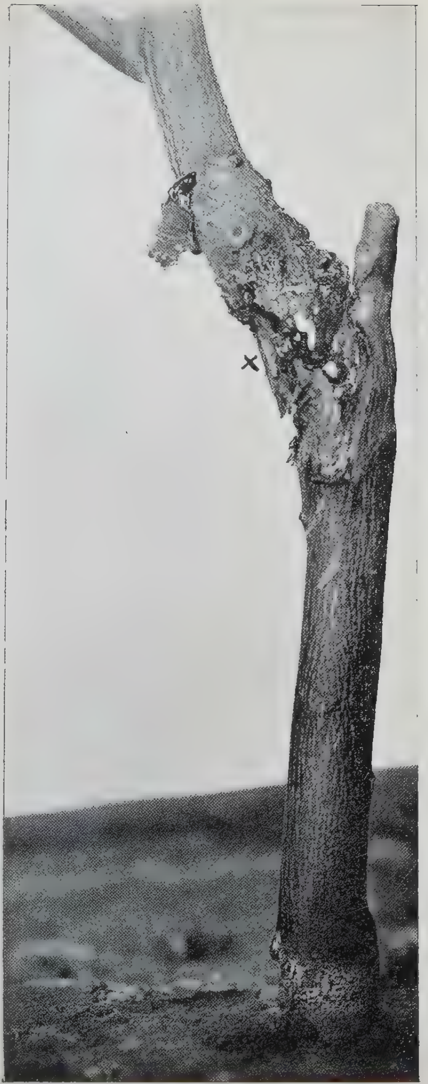


FIG. 1. A two-year old *mosambi* tree inoculated at bud union. Note active oozing out of gum at the inoculated spot marked "×".

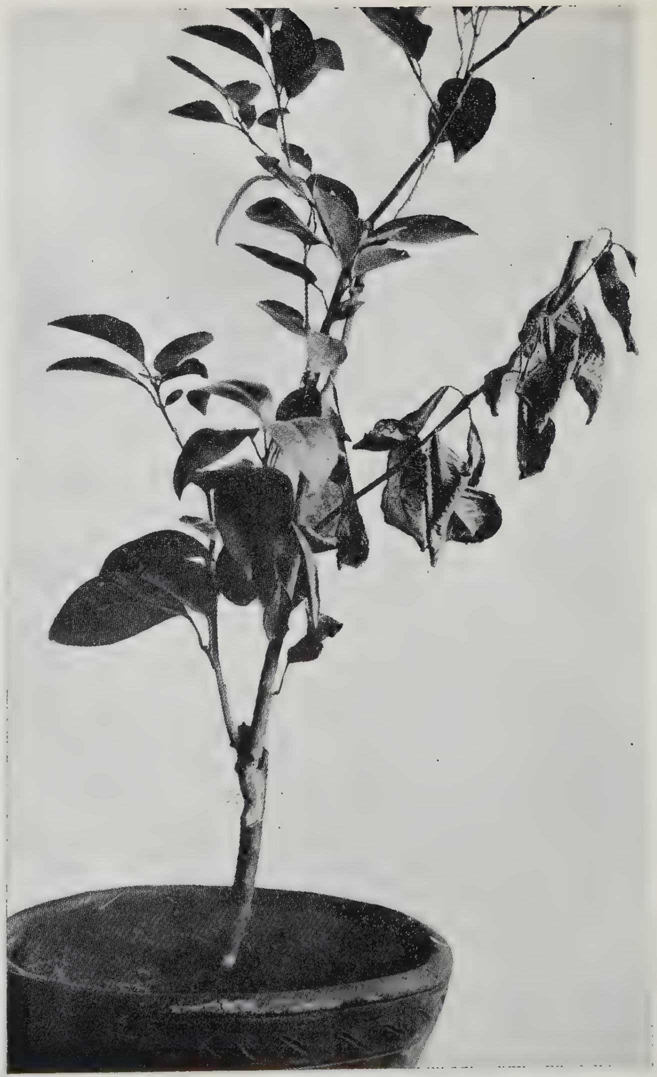


FIG. 2. An artificially infected *mosambi* tree showing yellowing and drooping of leaves on the inoculated side.

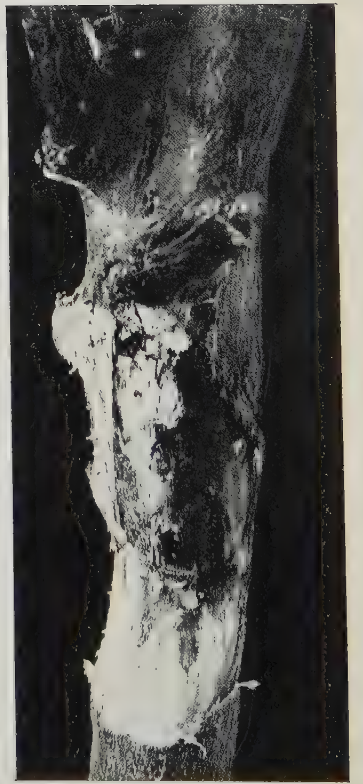


FIG. 3. Same as Fig. 2. showing the area of discoloured bark.

moist with sterile water. At the end of a week cotton was removed, and daily observations were recorded for the period of the experiment. Two of these experiments are described below.

On May 20, 1926, a two-year old *mosambi* tree budded on a *jamburi* stock, a horticultural variety of *Citrus limonia* Osbeck, was inoculated in the above manner at a point a little above the union. On May 31, gum drops appeared on the slit and the bark around the inoculated region had turned brown. After another week these symptoms became very pronounced until, on June 14, gum was actively oozing out of the slit (Plate XLVI, fig. 1), and the area of discoloured bark was $4\times1\frac{3}{4}$ cm. (Plate XLVI, fig. 3). The leaves on the inoculated side of the tree were yellow and drooping (Plate XLVI, fig. 2). A similar experiment was started on May 26, and the first signs of infection were visible on June 11. By June 15, gum was exuding copiously and the extent of discoloured bark was $3\times1\frac{1}{2}$ cm. The leaves on the inoculated side of the tree were drooping, whilst those on the opposite side were healthy. The fungus was recovered in re-isolation in both cases, and the control cuts remained healthy and healed normally without exhibiting any signs of infection.

Many similar inoculations were made, and the results of some of these tests are recorded in Table II. In all cases including those not mentioned in the table, inoculation with a pure culture of the fungus resulted in positive infection.

TABLE II

Results of inoculation of mosambi *trees with* Phytophthora *sp.*

Point of inoculation	Date of inoculation	Date of observation	Extent of gum lesion	Amount of gum formation
	1926	1926		
Bud union	20th May	24th July	6×3	Moderate
Bud union	1st June	20th Aug.	5×3	Moderate
Bud union	1st ,,	20th ,,	10×3	Abundant
Bud union	26th July	5th Oct.	4×2·5	Moderate
Bud union	26th ,,	26th Sept.	3×2	Moderate
Bud union	3rd Aug.	5th Oct.	5×3	Abundant
Bud union	26th July	5th ,,	6×2·5	Abundant
Bud union	3rd Aug.	5th ,,	4×3	Moderate
Bud union	6th Aug. 1928	10th Sept. 1928	4×3	Abundant
Bud union	6th Aug. 1929	10th Sept. 1929	12×3	Abundant
Controls	..	..	*Nil*	*Nil*

In another experiment the results of which are shown in Table III, inoculum was applied to slits made on different parts of a *mosambi* plant budded on a *jamburi* stock. The results indicate that the part of *Citrus sinensis* most susceptible to infection is the bud union, although inoculations also succeeded on aerial branches with slight gumming in a few cases. The roots and stock of *jamburi* were completely resistant, and this explains the common observation in the field that infection rarely travels downwards from the bud union.

TABLE III

Relative resistance of different parts of mosambi *to* Phytophthora *sp.*

Part of plant inoculated	Date of inoculation	Date of observation	Extent of lesion in cm.	Amount of gum formation
Stock, 4in. above ground	3rd Aug. 1926	5th Oct. 1926	2×1·5	*Nil*
Aerial branches	22nd Aug. 1928	10th Sept. 1928	*Nil*	Very slight
Stock, just below bud union.	3rd Aug. 1929	21st Aug. 1929	*Nil*	*Nil*
Roots just below the soil.	5th July 1926	25th Sept. 1926	*Nil*	*Nil*
Stock, 6 in. above ground.	27th July 1928	10th Sept. 1928	Very slight	*Nil*
Bud union	6th Aug. 1928	10th Sept. 1928	12×3	Abundant

RELATIVE RESISTANCE OF CITRUS TO GUMMOSIS

It is a common observation that different species of Citrus show variation in resistance to gummosis under field conditions. Some of the species are completely susceptible, whilst others exhibit resistance of a very high order almost approaching immunity. The information on the relative resistance of species of Citrus is therefore of great practical value, since the use of resistant stock is an important method of preventing gummosis.

The species and varieties of Citrus tested were those commonly grown or used for stock in the Presidency. Young plants of these species were grown in pots and inoculated with bits of mycelium inserted into slits cut in the bark and afterwards covered with wet cotton wool, which was removed after a week. The inoculated plants were kept in a shaded greenhouse and examined after definite periods when the length and width of discoloured bark were measured in each case. The results are summarised in Table IV.

TABLE IV

Relative resistance of different species of Citrus to mosambi Phytophthora

Species of Citrus inoculated	Point of inoculation	Date of inoculation	Date of observation	Extent of lesion in cm.	Amount of gum formation	Remarks
5-year old santra, *Citrus nobilis* var. *deliciosa*.	Bud union .	15th June 1926	20th Aug. 1926	2·5×1·5	Very slight	Strong tendency to be self-limited
5-year old santra, *Citrus nobilis* var. *deliciosa*.	Bud union .	15th June 1926	20th Aug. 1926	2·5×2	Very slight	Strong tendency to be self-limited
2-year old santra, *Citrus nobilis* var. *deliciosa*.	Bud union .	27th July 1928	25th Aug. 1928	Very slight	Very slight	No progress
2-year old pomelo, *Citrus grandis*.	Bud union .	27th July 1928	10th Sept. 1928	4×3	Abundant	Rapid progress
2-year old pomelo, *Citrus grandis*.	Stock .	22nd Aug. 1928	10th Sept. 1928	*Nil*	*Nil*	No infection
2-year old santra, *Citrus nobilis* var. *deliciosa*.	Just below bud union .	3rd Aug. 1929 .	26th Aug. 1929	*Nil*	*Nil*	No infection
2-year old santra, *Citrus nobilis* var. *deliciosa*.	Aerial branches.	22nd Aug. 1928	10th Sept. 1928	*Nil*	*Nil*	No infection
1-year old jamburi, *Citrus limonia*.	6 in. above ground.	27th July 1928	10th Sept. 1928	*Nil*	*Nil*	No progress; wound healed normally.
1-year old kagdi lemon, *Citrus aurantifolia*.	6 in. above ground.	27th July 1928	10th Sept. 1928	*Nil*	*Nil*	No infection
2-year old mosambi, *Citrus sinensis*.	Bud union .	3rd Aug. 1928 .	21st Aug. 1928	5×3	Moderate .	Rapid progress

The results indicate that the species of Citrus grown in Bombay can be grouped into three classes on the basis of their resistance to gummosis. The pomelo and *mosambi* are uniformly very susceptible, and in these species the lesion enlarges rapidly accompanied by profuse gumming. The *mosambi*, *Citrus sinensis* Osbeck, showed such a high degree of susceptibility to gummosis that the area of the lesion was 5×3 cm. in eighteen days after inoculation. Next in the order of resistance is the common mandarin or *santra*, *Citrus nobilis* Lour. var. *deliciosa* Swingle, in which the extent of the lesion produced is limited and there is very slight gum formation ; the inoculated trees show a strong tendency to form a callus tissue along the edges of the dead bark. The *kagdi* lemon, a horticultural variety of *Citrus limonia* Osbeck, may be considered almost immune from gummosis, since the disease makes little progress and there is rapid callus formation and the wound heals completely. These results are thus in accord with the general horticultural practice to graft the sweet susceptible *mosambi* on the sour resistant stock of *jamburi*.

THE CAUSAL FUNGUS

Morphology.—The mycelium is unseptate, sparingly branched and granular. On corn-meal agar the mycelium makes moderate aerial growth. Within the rind the mycelium is not so irregularly shaped as on culture media, and is mostly inter-cellular, sometimes intra-cellular without haustoria (Plate XLV, fig. 3D). On potato-dextrose agar there is practically no aerial growth, but the mycelium within the medium is stout and irregularly shaped. On oat-meal agar there is also little aerial growth but better than on any of the media tested.

The relation of temperature to growth on oat-meal agar was tested, and cultures were incubated at 4·5, 7·5, 11·5, 17, 20, 26-27, 30, 32·5 and 35° C. in the cold storage rooms of the Fruit Research Station at Ganeshkhind. The tests were run in triplicates, and the results in Table V are the average of the growths in the plates incubated at each of the above temperatures. Growth was measured after eight days.

TABLE V

Effect of temperature on growth of mosambi Phytophthora *on oat-meal agar*

Temperature (°C.)	Mean diameter of colonies in cm.
4·5	0
7·5	trace
11·5	1
17	6.9
20	8.5
26-27	9.7
30	9.3
32·5	4.5
35	0

The fungus grew abundantly at 27 to 30° C., but failed to develop at 35° C. At lower temperatures, it made very slight growth at 7·5° C., but failed to grow at 4·5° C. The rate of growth at different temperatures is illustrated in Fig. 1.

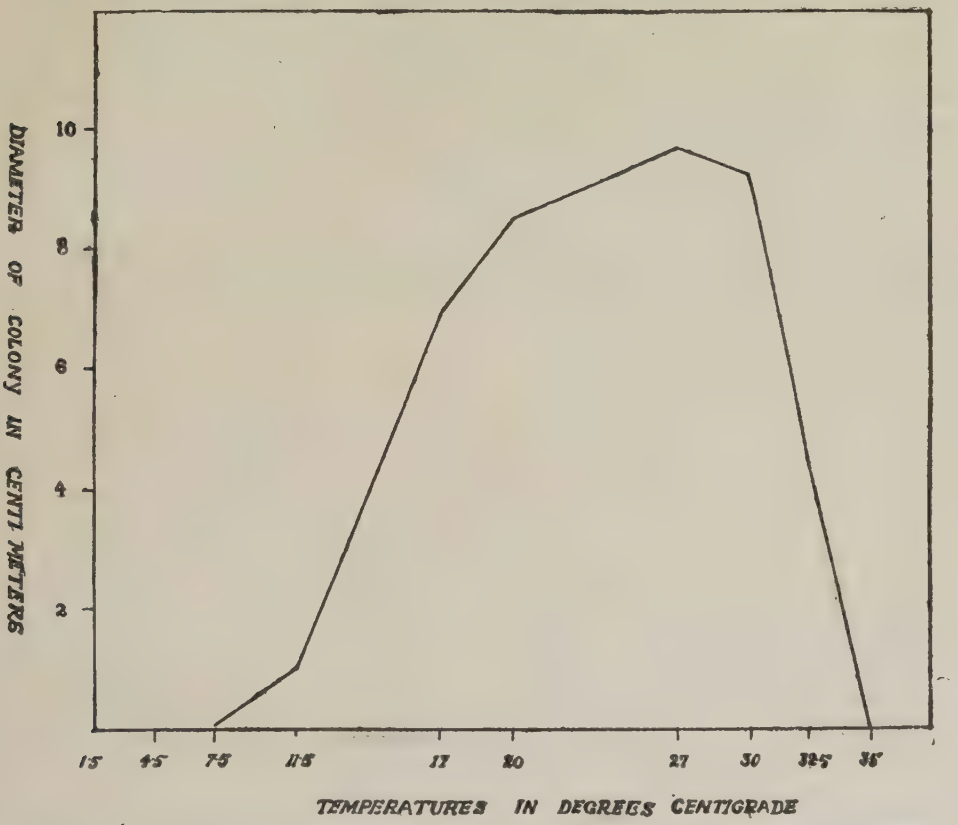


Fig. 1. Showing the effect of temperature on the growth of *mosambi Phytophthora* on oat-meal agar.

Sporangia are completely absent on potato-dextrose agar, but are fairly abundant on one-week-old cultures of oat-meal agar. Corn-meal agar was not as favourable for sporangium formation as oat-meal. The sporangia are large, terminal and lemon-shaped with a prominent apical papilla of dehiscence (Plate XLV, fig. 3 A). They are extremely variable in size and measure from 23 to 79μ in length, with the length mode falling in the class between 53 to 54·9. The range of variation in width, however, is not large, since about seventy per cent of sporangia fall in the class between 25 and 28·9μ (Table VI).

TABLE VI

Measurements of sporangia of Phytophthora *causing gummosis of* mosambi

Length		Width	
Classes in μ	No. of sporangia in 400	Classes in μ	No. of sporangia in 400
21 to 22·9	0	15 to 16·9	0
23 to 24·9	2	17 to 18·9	9
25 to 26·9	3	19 to 20·9	10
27 to 28·9	6	21 to 22·9	22
29 to 30·9	8	23 to 24·9	31
31 to 32·9	8	25 to 26·9	160
33 to 34·9	9	27 to 28·9	119
35 to 36·9	9	29 to 30·9	27
37 to 38·9	10	31 to 32·9	14
39 to 40·9	10	33 to 34·9	8
41 to 42·9	11	35 to 36·9	0
43 to 44.9	14		
45 to 46·9	15		
47 to 48·9	23		
49 to 50·9	41		
51 to 52·9	54		
53 to 54·9	60		
55 to 56·9	29		
57 to 58·9	20		
59 to 60·9	17		
61 to 62·9	16		
63 to 64·9	10		
65 to 66·9	7		
67 to 68·9	7		
69 to 70·9	4		
71 to 72·9	3		
73 to 74·9	2		
75 to 76·9	1		
77 to 78·9	1		
79 to 80·9	0		

Sporangia are produced sparingly in culture, but they are formed freely when young mycelium from a rich medium, such as prune juice, is transferred to sterile blotting paper floating in water in a Petri dish. Under these conditions sporangia are produced in abundance within twenty-four hours. Recently Fawcett and Klotz [1934] have described a somewhat similar method for inducing abundant production of sporangia in *Phytophthora.*

Germination of mature sporangia was studied in water cultures and is much influenced by temperature. The figures in Table VII are the average of three tests.

TABLE VII

Effect of temperature on germination of sporangia of mosambi Phytophthora

Temperature (°C.)	Per cent of germination	
	Direct	Indirect
1·5	0	1·2
4·5	0	6·3
7·5	3·1	15·3
11·5	6·9	33·9
17	12·7	48·6
20	16·1	54·7
26-27	47·6	14·7
30	61·9	4·7
35	20·3	0

The sporangia produce zoospores at 1·5°C., and the amount of indirect germination increases as temperature rises, and is at its optimum at 20°C. ; it falls off sharply with further rise in temperature until it is replaced completely by direct germination at 35°C. The sporangia also germinate by the production of germ-tubes, and the optimum temperature for this type of germination is 26-27°C. They do not produce germ-tubes below 7·5°C., but do so readily at 35°C., at which temperature the fungus fails to make any mycelial growth on oat-meal agar (Fig. 2).

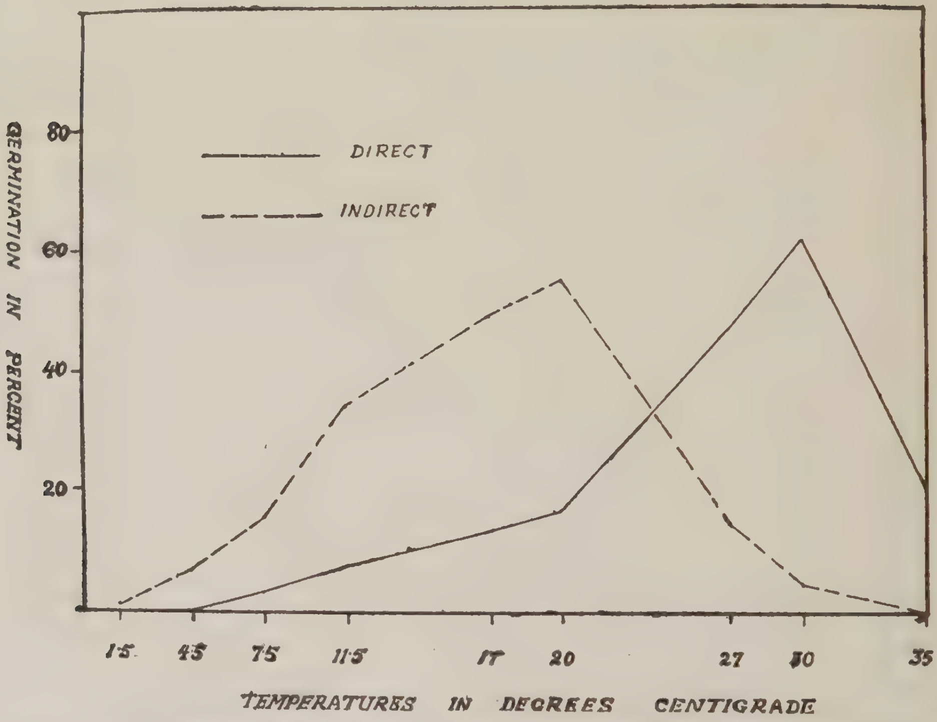


Fig. 2. Curve showing the effect of temperature on the germination of the sporangia of *mosambi* Phytophthora in water cultures.

The fungus does not form oogonia in culture, but produces chlamydospores in large numbers in cultures about two weeks old on oat-meal or corn-meal agar. They are pale yellow, spherical or sub-spherical, mostly terminal but sometimes intercalary, thick-walled and granular, and germinate by one or more germ-tubes (Plate XLV fig. 3 B). In size they range from 12 to 43μ, a majority falling in the class between 29 to 35μ (Table VIII).

TABLE VIII

Measurements of chlamydospores of mosambi Phytophthora

Classes in μ	Diameter
	Number of chlamydospores in 400
11 to 12·9	0
13 to 14·9	1
15 to 16·9	11
17 to 18·9	13
19 to 20·9	14
21 to 22·9	16
23 to 24·9	28
25 to 26·9	30
27 to 28·9	30
29 to 30·9	50
31 to 32·9	97
33 to 34·9	61
35 to 36·9	29
37 to 38·9	17
39 to 40·9	2
41 to 42·9	1

TAXONOMY *

In connexion with the identity of this fungus the species of *Phytophthora* that bear close resemblance to the description above given are *Phytophthora citrophthora* (Sm. & Sm.) Leonian, *P. parasitica* Dast. emend. Ashby and *P. Palmivora* Butler. *Phytophthora citrophthora* is very similar to the *mosambi Phytophthora* found in Bombay in respect to the morphology of sporangia and chlamydospores and the type of growth on culture media, but differs from the latter by its lower temperature range, the optimum being between 25° and 27°C. (1931). Moreover, unlike the *mosambi Phytophthora, P. citrophthora* does not grow at 32·5°C. but makes slight growth at 5°C. . The *mosambi Phytophthora* in Bombay thus differs from *P. citrophthora* in its ability to grow at higher temperatures, a character considered by Tucker [1931] as of specific value and a useful adjunct to morphological characters in the taxonomy of the genus.

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* The writers are grateful to Mr. S. F. Ashby, Director, Imperial Mycological Institute, London, for help in the identification of the fungus.

However, the position in respect to temperature relations is reversed when the *mosambi Phytophthora* is compared with *P. parasitica*. The latter develops rapidly at 35°C. (1931), at which temperature the growth of the *mosambi Phytophthora* is completely suppressed. On culture media the *mosambi Phytophthora* makes moderate aerial growth, whilst *P. parasitica* grows very profusely (1931). Besides, the tufted growth of *P. parasitica* serves to distinguish it from the radiate and appressed growth characteristic of *mosambi Phytophthora* (1931).

It will be seen from the above discussion that the *mosambi Phytophthora* can be readily separated from *P. parasitica* on the basis of its inability to grow at 35°C. and the radiate character of its growth. It, however, makes a fairly good growth at 32·5°C., and in this respect resembles *P. palmivora* and differs from *P. citrophthora* ; its optimum temperature for growth also approximates that of *P. palmivora* and is higher than that of *P. citrophthora*. The abundant formation of chlamydospores and in a few cases of sporangia in young cultures and the conidial dimensions are the other two characters which the isolation from *mosambi* has in common with a typical strain of *P. palmivora*. Also like *P. palmivora*, the Bombay fungus does not form oospores in pure culture and, according to Mr. S. F. Ashby to whom a culture was sent for opinion, it falls in the ' rubber ' group of *P. palmivora* as it readily forms these sexual bodies when grown with a member of the ' cacao ' group.

Phytophthora palmivora has not been reported elsewhere* as the cause of gummosis in citrus although Tucker [1931] is of the opinion that this species has probably a wide geographic distribution, and that many of the isolations from citrus in various regions generally referred to *Phytophthora citrophthora* are probably strains of *P. palmivora*. In a letter Mr. Ashby also reports that an identical strain has been isolated by Miss Hellinger from a brown lateral lesion on an orange fruit from Tanganyika Territory in East Africa. It appears, however, that the strain causing gummosis and brown rot of *mosambi* in Bombay is distinct from the one shown by Ocfemia and Rolden [1927] to cause seedling and shoot blight of various species of Citrus in the Philippines.

CONTROL

Prevention.—In the control of citrus gummosis, preventive measures play an important part, and some of the methods in common practice may be briefly discussed.

It has been shown above that some of the sour varieties of citrus, notably *jamburi*, a horticultural variety of *Citrus limonia*, are highly resistant to gummosis, and that the causative fungus enters the tree at the bud union. It is therefore essential to graft pomelo or *mosambi* on a resistant stock at least six inches above the ground. This method was first introduced in Italy and is now commonly practised in California and other citrus-growing regions.

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* After the paper was submitted for publication, Briton-Jones and Baker reported *Phytophthora palmivora* as the cause of gummosis in citrus in Trinidad. (*Tropical Agriculture* **12** : 119-125. 1935).

Soil should not be piled up around the trunk to prevent the collar from too long contact with irrigation water. This practice is very important in orchards where citrus trees are budded low or planted deep in the soil, since the causal organism enters readily at the bud union from the soil harbouring infection. The earth should therefore be removed at the base of the trunk and piled into a small encircling mound so as to prevent both soil and irrigation water from coming into direct contact with the trunk.

Excessive flooding of gardens should be avoided as this tends to create water-logged conditions in heavy soils.

Treatment.—When citrus trees are affected, the only feasible method of treating gummosis is the excision of the diseased bark. Treatment, however, is difficult if the disease is in an advanced stage, although gummosis can be successfully treated in early stages. Before describing experiments on the effectiveness of fungicides tested, the method of treatment may be briefly discussed.

The bark should be scraped until the extent of the diseased patch is determined. The affected bark should then be isolated by cuts extending to the wood, taking care that no injury is caused to the wood by this operation. The cuts should be extended at least half an inch on all sides beyond the limits of the diseased zone. The affected bark should then be removed and the wound covered with twenty-five to thirty per cent creosote oil, which should be allowed to penetrate into the wood and the bark at the sides of the wound so as to kill the fungus. After the surface of the wound is dry, it may be painted with coal tar to protect the exposed wood from the wood-rotting fungi.

If the diseased bark is not carefully removed, the disease may re-appear at the side of the wound, which will require further treatment. Growers are therefore advised to inspect the treated trees periodically to see whether the treatment has been successful.

FUNGICIDES

These experiments were undertaken in the search of a fungicide which, besides being a good disinfectant, is easy to apply and penetrates readily into the wood and the bark at the cut surface of the wound. A brief description of these experiments follows.

Experiments in 1924-26.—The fungicide tested was crude liquid carbolic acid diluted with an equal amount of water, and the results have already been reported separately by one of us [Kamat, 1927], but are briefly summarised here to facilitate reference. About 133 diseased *mosambi* trees were treated in 1924-25, out of which 120 trees were completely cured. The appearance of gummosis in a few cases was probably due to the failure to remove diseased bark completely. The results are shown in Table IX.

TABLE IX

Experiments on the treatment of gummosis with 50 per cent carbolic acid during 1924-26

Place	Number of trees treated	Number of trees cured	Per cent of success
	1924-25		
Baramati	60	55	91·6
Poona	73	65	89·0
	1925-26		
Junnar	201	200	99·5
Rahata	252	245	95·2

Since carbolic acid seemed promising, extensive trials were made in 1925-26 at Junnar and Rahata. The results in Table IX will show that 444 trees completely recovered out of 453 trees treated for gummosis.

Experiments in 1928-29.—Since carbolic acid was not injurious to the bark when diluted with water, its use was safely recommended to citrus growers in the Presidency. In 1928, however, one of the prominent growers in East Khandesh suffered very heavy losses due to the death of many hundred *mosambi* trees caused by the application of undiluted crude liquid carbolic acid. This fungicide was therefore considered entirely unsuitable in the hands of citrus growers, and the search was again made for an effective treatment incapable of causing injury to the treated trees under any conditions.

The fungicides selected for field trials were Zulite, Florium, Coal-tar Creosote and crude liquid carbolic acid. Zulite and Florium were proprietary compounds and were sent for testing by a commercial firm in Bombay. Creosote and Zulite being insoluble in water were applied in full strength. Florium, however, was used in dilute solutions and also in full strength. Carbolic acid was used as a check. All treatments were made in duplicate, but one set of treated trees was painted with coal tar after the treatment and the other was left uncoated. The results of the field trials in six different centres are recorded in Table X.

TABLE X

Summarised results of experiments with different fungicides in the treatment of gummosis during 1928-29

Treatment	Number of trees treated	Number of trees cured	Per cent of success
Zulite	16	6	37·5
Zulite and tar . . .	13	4	30·8
Creosote	17	4	23·5
Creosote and tar . . .	13	7	53·8
Florium	13	4	30·8
Florium and tar . . .	12	6	50·0
50 per cent Florium . .	12	5	41·6
50 per cent Florium and tar .	10	6	60·0
25 per cent Florium . .	10	7	70·0
25 per cent Florium and tar .	8	7	87·5
50 per cent carbolic acid .	9	3	33·3
50 per cent carbolic acid and tar	9	3	33·3
25 per cent carbolic acid .	8	4	50·0
25 per cent carbolic acid and tar	9	3	33·3

These results did not indicate any special advantage for any of the fungicides tested over the standard carbolic acid treatment. Dilute solutions of Florium were, however, the least satisfactory.

Experiments in 1929-30.—Since the results obtained in the last year were inconclusive, field trials were continued at Rahata with Florium, Zulite and Creosote oil, which latter is a proprietary compound containing twenty-five to thirty per cent high-boiling compounds of coal tar and was supplied by the Eastern Chemical Company, Bombay. The treated trees were about eight to ten years old. The results are recorded in Table XI.

TABLE XI

Experiments on the treatment of gummosis with different fungicides during 1929-30

Treatment	Number of trees treated	Number of trees cured	Per cent of success
Creosote oil	7	7	100
Zulite and tar . . .	7	3	42·8
50 per cent Florium and tar .	7	5	71·4
25 per cent Florium and tar .	11	8	72·7
50 per cent carbolic acid and tar	10	10	100
25 per cent carbolic acid and tar	10	10	100

Zulite and Florium were again relatively ineffective, but all trees treated with Creosote oil recovered completely. It appears that Creosote oil has good penetrating quality and kills the fungus in the bark at the margin of the wound. It has still another advantage in that the wound covered with Creosote oil does not require to be painted with any other substance.

Experiments in 1930-31.—The results with Creosote oil (25 to 30 per cent) were so promising that it was decided to give this fungicide an extended trial with many other fungicides including four new compounds, namely wood-tar Creosote, Anti-ant, and Pesterine and Colas mixed in equal proportions. Wood-tar Creosote is a product of the Mysore Iron Works derived as a bye-product in the distillation of alcohol from wood. Anti-ant was a proprietary compound supplied by Hardcastle, Waud & Co., Bombay, and was mixed with four parts of kerosene oil before applying to the gummous wound. Pesterine and Colas were obtained from the Burmah-Shell Company, Bombay. Through the generous co-operation of Sheth Manakchand Kisondas, about 300 gum-affected trees were placed at our disposal for treatment in his plantation at Utran in East Khandesh. The new fungicides were also tested at Varkhedi. The results are summarised in Table XII.

TABLE XII

Experiments on the treatment of gummosis with different fungicides during 1930-31

Treatment	Utran		Varkhedi	
	No. of trees treated	Per cent of trees cured	No. of trees treated	Per cent of trees cured
Creosote oil	54	98·2	45	89
Creosote oil and tar	32	100	11	91
Wood ar creosote	13	77	43	44
Woodtar creosote and tar	..	..	10	70
Pasterine and Colas (1 : 1)	5	80	58	38
Anti-ant	..	..	126	91·3
Florium	11	82	..	..
Florium and tar	5	80	..	..
50 per cent Florium	5	80	..	..
50 per cent Florium and tar	4	75	..	..
25 per cent Florium	4	50	..	..
25 per cent Florium and tar	4	75	..	..
Zulite	32	90·7	..	..
Zulite and tar	30	93·4	..	..
50 pe cent carbolic acid	47	93·7	..	..
50 per cent carbolic acid and tar.	26	92·4	..	..
25 per cent carbolic acid	18	94·5	..	..
25 per cent carbolic acid and tar.	10	90	..	..

The results of trials at Utran bring out the high fungicidal value of Creosote oil, in which case only one tree failed to recover out of eighty-six trees treated with this fungicide. They also confirm the observation that painting with coal tar of wounds covered with Creosote oil can be dispensed with without serious disadvantage. The results of trials at Varkhedi, however, have little significance since the trees were badly affected before the treatment was applied, and in some cases the disease had almost girdled the trees.

Experiments in 1931-32.—Encouraged by the results obtained during the last year, another series of trials was arranged at Belapur and Rahuri with Creosote oil, woodtar creosote, Anti-ant and Pesterine mixed with Colas. The results in Table XIII are in accord with those reported in the previous experiments and clearly establish the superiority of Creosote oil as a fungicide.

TABLE XIII

Experiments on the treatment of gummosis with different fungicides during 1931-32

Treatment	Belapur		Rahuri	
	No. of trees treated	Per cent of trees cured	No. of trees treated	Per cent of trees cured
Creosote oil	65	100	17	100
Woodtar creosote . . .	..	..	33	2·8
Pesterine and Colas (1 : 1) .	..	..	12	83·4
Anti-ant	28	100	..	..

SUMMARY AND CONCLUSIONS

Gummosis produces typical symptoms in *mosambi*, *Citrus sinensis* Osbeck, and affects the trunk. It is characterised by the copious exudation of a resinous gummy substance and the cracking of the bark for considerable distances upwards from the bud union. The serious aspect of the disease is, however, in the lateral extension of the invaded region, which, if not treated, will kill the tree. The causative fungus also attacks *mosambi* fruits (and *santra* fruits lying on the ground) causing brown rot ; the diseased *mosambi* fruits fall to the ground and are reduced to a mass of putrefying tissue through the invasion of secondary decay organisms.

The disease is widely distributed in the *mosambi* orchards in the Bombay-Deccan, but has not been reported elsewhere in this country. The disease spreads rapidly during damp or wet weather.

Gummosis can be produced by inoculating bits of mycelium from a young culture of *Phytophthora* isolated from an infected fruit, into slits made in the cortical tissue of the stem.

Experiments on the relative resistance of different species of Citrus indicate that the pomelo and *mosambi*, *Citrus sinensis*, are highly susceptible, whilst the *kagdi* lemon, a horticultural variety of *C. aurantifolia*, and *jamburi*, a horticultural variety of *C. limonia*, are almost immune from gummosis. The common mandarin or *santra*, *C. nobilis* Lour. var. *deliciosa* Swingle, occupies an intermediate position.

The morphology of the causative fungus has been described in detail, and the fungus is further compared with *Phytophthora citrophthora* and *P. parasitica*, the cause of citrus gummosis in other countries. It has been shown that the *Phytophthora* causing *mosambi* gummosis in Bombay is a typical strain of *Phytophthora palmivora* Butler in the 'rubber group' of that species.

Grafting of sweet, commercial varieties of citrus on a sour, resistant stock, such as *jamburi*, will prevent gummosis. Both soil and irrigation water should not be allowed to come into direct contact with the bud union, since the fungus enters the cortical tissue at the bud union from the soil harbouring infection.

The relative effectiveness of a number of fungicides in treating gummosis has been tested. The best method of treatment is to remove the bark and to cover the wound with twenty-five to thirty per cent Creosote oil ; the wound may then be painted with coal tar, if necessary.

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A NOTE ON THE HAIRINESS OF COTTON

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(Received for publication on 4th February 1936)

The degree of hairiness of cotton is of considerable importance as it has to be " critically studied in the isolation of special varieties and races of cotton "*. Moreover presence or absence of hairs is thought to be associated with resistance or susceptibility to various insect pests, such as Jassids (*Empoasca devastans*), cotton stem borer, etc. Hairy cottons are also supposed to be resistant to Red Leaf Blight in southern India. In the Punjab it has long been known that hairy cottons are to be preferred for growing in localities where Jassids are a serious pest. The investigation of the degree of hairiness of various varieties of cotton is, therefore, of considerable importance.

Indigenous varieties of cotton grown in the Punjab are very hairy indeed, but the acclimatized American strains differ a great deal in hairiness. It is realized that it is not very safe to introduce smooth-leaved American varieties for general cultivation in the Punjab. The determination of the degree of hairiness of all American strains bred at the Cotton Research Station, is, therefore, a routine operation of great importance. During these investigations, certain observations have been made, which, so far as the writer is aware, have not been previously recorded. These will be briefly described in the present note.

PREVIOUS LITERATURE

Before describing the observations made, the previous literature will be briefly reviewed. References to hairiness of cotton are met with mostly in the genetic studies of this plant. Balls [1912] records that hairiness is of several types and is controlled by a number of factors. Kearney [1923] found that the degree of hairiness of petiole was highly correlated with that of the lower surface of the leaf. He observed that the young leaves were much more hairy than the mature ones and in his genetic studies he recorded the hairiness of half mature leaves. Kulkarni and Khadilkar [1929] also studied the inheritance of hairiness in a cross between *G. hirsutum* and *G. barbadense*. They have given rather extensive data on this point, but have not attempted any factorial analysis of the figures. The method of recording the degree of hairiness has also not been given. Harland [1932] has studied the inheritance of hairiness in several species of cotton. He found intimate connection between the hairiness of various organs of the plant.

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*" Wild and cultivated cotton plants of the World," by Sir George Watt. Longmans Green and Company, London, 1907 ; p. 59.

From this review of literature it is found that in genetic studies the method of recording the degree of hairiness has not been mentioned by many workers. This, however, is a very important point as will be shown in discussing the present data and it is surmised that by a proper appreciation of the facts brought out here, the analysis of hybrid population for genetic studies will be greatly facilitated.

MATERIAL AND METHOD

The degree of hairiness of all important new strains of American cotton has been recorded. During 1933 the leaves of various varieties were only examined, but during 1934 the hypocotyledonary region and the cotyledons were also kept under observation.

In the case of hypocotyledonary region small areas of the bark were peeled off and an area of one cm. ×1·5 mm. just from below the cotyledonary node was examined under the microscope. Five such areas from different plants were examined before the shedding of the cotyledons and the hairiness was expressed as an average of these five readings. Thus twelve varieties of American and one of *desi* cottons were examined. All American varieties without any exception were found to be non-hairy, while the *desi* variety (15 Mollisoni) had eighty-seven hairs per 15 sq. mm.

The cotyledons were examined under a binocular and both surfaces were scrutinized in all the varieties mentioned above. It was found that both surfaces of the cotyledons of all varieties, including the *desi* which has harirs on the hypocotyledonary region, were absolutely glabrous. This is rather surprising as the veins and leaves of some of these varieties are exceedingly hairy.

It has been reported that hairs on stem and leaves of cotton are deciduous. It was, therefore, thought necessary to fix the age at which counts should be made. It was decided that all counts should be taken at the age of twenty-two days on which day the leaf is fully mature [Afzal, 1933]. It was also considered necessary to confine our attention to the leaves borne directly on the main stem. Five leaves from five different plants were taken at each time and the number of hairs was counted from an area of one sq. cm. from five different places on each leaf and the hairiness was expressed as an average of all these figures.

In order to study the hairiness of a large number of varieties, counts were made only on the 5th, 15th, 25th and 35th leaf on the main stem. The figures are given in Table I. A consideration of these figures reveals that the leaves produced higher up on the main stem are more hairy than those produced lower below. This point is very important as it might afford an explanation of the confusion of the genetic data with regard to hairiness. It is quite conceivable that the hairiness of different plants in various progeny rows was not counted on leaves of similar ages and similar situation on the main stem. This error alone would cause enough discrepancies in the data as to render these unsuitable for factorial analysis.

TABLE I

Average number of hairs per sq. cm. of leaf area

—	5th leaf				15th leaf				25th leaf				35th leaf			
	1933		1934		1933		1934		1933		1934		1933		1934	
	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral
(1) 4 F	41	41	50	8	494	72	369	99	918	145	805	385	898	257	737	391
(2) 43 F	38	31	26	10	428	169	575	185	630	173	638	241	1272	309	621	232
(3) 38 F	10	7	Leaves dried up		107	15	46	16	389	76	445	131	..	..	459	102
(4) 45 F	..	..	4	3	..	..	28	12	..	..	349	79	..	..	457	140
(5) L. S. S.	..	..	17	6	..	..	393	77	..	..	632	127	..	..	516	123
(6) 289 F	13	11	..	..	112	27	..	..	152	70	..	..	625	73	..	..
(7) N. T. 4 F	18	11	..	..	212	43	..	..	563	71	..	..	599	112	..	..
(8) 1 F	20	8	..	..	197	49	..	..	527	117	..	..	384	56	..	..
(9) 48 F	6	6	..	..	117	25	..	..	374	88	..	..	691	154	..	..
(10) M. P. 289 F	13	11	..	..	91	53	..	..	554	43	..	..	398	147	..	..
(11) K. T. 23	2	1	..	..	121	25	..	..	449	77	..	..	512	65	..	..
(12) K. T. 25	5	3	..	..	13	4	..	..	139	6	..	..	270	37	..	..
(13) 15 Mollisoni	491	392	306	215	693	465	808	487	1396	1135	1079	943	1390	503	1169	746

These figures are, of course, from a few isolated leaves on the main stem and in order to confirm these, counts were made on all the successive leaves. Five plants of 4 F were examined on 27th-29th September 1934 and the figures are given in Table II. It will again be seen that the upper leaves are much more hairy than the lower ones.

TABLE II

Average number of hairs per sq. cm. of leaf area in successive leaves up the main stem

Leaf No.	15 Mollisoni		43 F		4 F			
	1933		1933		1933		1934	
	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral
26							518	61
27							559	84
28							587	86
29							544	87
30			477	174			623	105
31							654	117
32							629	110
33							685	109
34							719	128
35			408	109			744	142
36					773	122	777	152
37							679	138
38					760	127	756	155
39			504	194			823	196
40					882	122	886	229
41			494	137	713	111	986	266
42			765	187	1156	142	981	339
43			Innumerable				1068	399
44	1859	1226						
49	1224	890						
54	2077	1294						
59	2796	1736						
64	innumerable							

The relative hairiness of different varieties is also clear from Table I. The *desi* variety, 15 Mollisoni, is very hairy indeed.

Counts of Jassid population were also taken in a few cases and it appears that some correlation does exist between the hairiness of leaf and Jassid population.

SUMMARY

The hypocotyledonary region of all American strains grown in the Punjab was found to be glabrous, but that of *desi* cotton had eighty-seven hairs per 15 sq. mm. The cotyledons of all varieties were devoid of hairs.

It has been found that the degree of hairiness increases from the bottom of the plant to the top in all varieties. It is, therefore, pointed out that in all genetic studies on hairiness of cotton, it is necessary to fix the location of the leaf and its age for hair-counting in a hybrid population, otherwise genetic analysis would be rendered difficult.

ACKNOWLEDGMENTS

This work was carried out as a part of the Punjab Botanical Research Scheme financed jointly by the Indian Central Cotton Committee and the Punjab Government.

I wish to acknowledge the help of my Assistants Messrs. Sant Singh Verma and B. K. Kar in carrying out these studies.

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A NOTE ON THE STERILE HYBRID BETWEEN *NICOTIANA TABACUM* L. AND *N. PLUMBAGINIFOLIA* VIV.

BY

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(Received for publication on 31st March 1936)

(With Plate XLVII and one text-figure)

Introduction

The genus *Nicotiana* has been used very extensively for genetic investigations. With the object of extending knowledge on the compatibility and relationships of the numerous species, a large number of crosses have been made between them by various workers. One of the most widely used species has been *N. Tabacum* probably because it crosses so readily and also perhaps because of its economic importance. East [1928] in his comprehensive account of the genetics of this genus states that one of the few instances where an attempt to cross *N. Tabacum* with another species has resulted in complete failure is that involving *N. plumbaginifolia*. Christoff [1928] obtained a few seedlings from the cross *N. Tabacum* var. *macrophylla* × *N. plumbaginifolia* but they died after producing 4-5 leaves. The reciprocal cross was entirely unsuccessful. Despite these failures, it seemed desirable to attempt the cross again as a study of the important " leaf-curl " disease of tobacco at Pusa indicated that out of a number of species and varieties, only *N. plumbaginifolia* possessed any resistance or tolerance to the disease. If this species could be crossed with a commercial variety of tobacco, then there was hope that it might be possible to transfer the disease-resistance of the former to the latter. The endeavour to cross *N. plumbaginifolia* with the Pusa type H. 156 proved successful and three vigorous hybrid plants were raised to maturity.

It should be pointed out, however, that *N. plumbaginifolia* is closely allied to *N. longiflora* Cav. and is considered by some authors [East, 1928; Hollingshead, 1929] to be merely a variety of the latter. *N. longiflora* has been crossed with *N. Tabacum* by Christoff [1928] who obtained only defective seedlings, and by Malloch and Malloch [1924] who obtained a number of seedlings one of which survived long enough to produce flowers. The latter, however, according to the authors' statement, was a weakly plant quite different from the vigorous *Tabacum-plumbaginifolia* hybrids which form the subject of this note. These are sufficiently interesting, in our opinion, to merit a brief description.

The cross was " difficult " and a very large number of pollinations had to be made. *N. plumbaginifolia* was used as the male parent. Two hundred and thirty-nine capsules containing much shrivelled seeds were finally obtained. The seeds were sown in flats in August 1934, and seven seedlings raised therefrom.

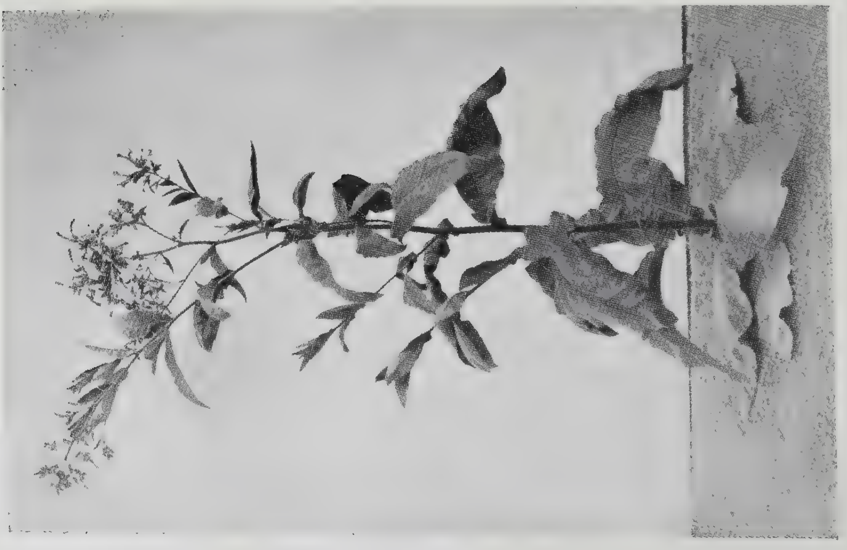

FIG. 1. *N. Tabacum* var. Pusa H. 156

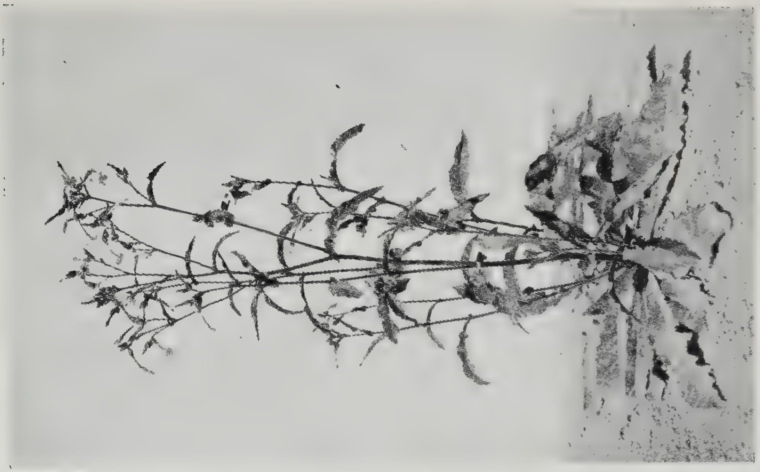

FIG. 2. *N. Tabacum* × *N. plumbaginifolia*—F_1

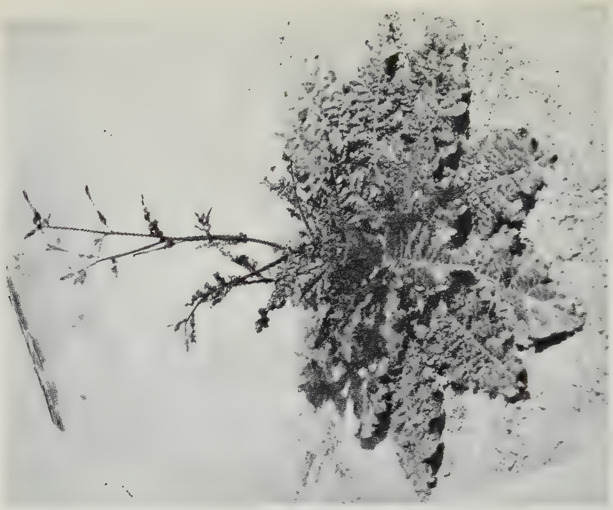

FIG. 3. *N. plumbaginifolia*

The latter were planted out in the field when large enough to handle. They showed marked differences in vigour, and only three reached maturity. These, however, were vigorous, and flowered very freely.

DESCRIPTION OF PARENT VARIETIES AND F_1

N. Tabacum *var. Pusa H. 156 (Plate XLVII, fig. 1)*

Habit.—Plants tall, about 200 cm. high, with long internodes ; leaves distributed on the stem at regular intervals.

Leaves.—Sessile, amplexicaul, inserted at an angle of about 75° with the stem ; auricles broad and decurrent ; lamina ovate ; margin entire or slightly wavy ; apex acute ; surface smooth ; texture soft ; midrib narrow at base, hairy, smooth ; hairs on midrib of one kind with terminal, unicellular, bluntly pear-shaped glands. Foliage colour light green.

Inflorescences.—Terminal open cymes.

Flowers.—Large, remaining open day and night. Pedicel persistent, about 2·0 cm. long, light green in colour. Calyx loose, dilated, more or less globose, light green ; 5-fid, lobes triangular, unequal and pointed ; ridges not present on calyx tube. Corolla tube broad, abruptly dilating from about the middle ; colour white ; 5-fid, lobes broadly triangular and faintly marked by a mid-vertical line ; orifice about 1·3 cm. in diameter. Inside of corolla base densely hairy. Stamens five, epipetalous ; filaments long, fused at base only ; anthers large, oval, green.

Ovary conical, green. Style about 4·0 cm. long, thick, usually reaching the level of anthers but remaining a little below the orifice in open flowers. Stigma large, bi-lobed.

N. plumbaginifolia *(Plate XLVII, fig. 3)*

This species is found as a common weed in several parts of India. There appears to be a considerable variation between the different forms. The form used in this investigation is described below.

Habit.—Plants short, about 60 cm. high, with a compact basal rosette of leaves which lie very flat on the ground, throwing up a central floral axis.

Leaves.—Sessile, non-auriculate ; lamina lanceolate ; margin very wavy ; apex acute ; surface corrugated ; texture hard ; midrib broad at base, hairy, rough. Hairs of three kinds present, *viz.*, (*a*) hairs glandular at base, (*b*) hairs with terminal, unicellular globose gland, (*c*) small non-glandular hairs. Foliage colour dark green.

Inflorescence.—A central axis terminated by a very loose cymose inflorescence.

Flowers.—Small, persistent, opening only at night. Pedicel about 0·9 cm. long, purple. Calyx closely enclosing base of corolla tube, dark green with deep purple pigment at base and at tips of lobes ; 5-sect, lobes narrow, more or less linear, equal ; ten prominent ridges present.

Corolla long, narrow, dilating slightly near the mouth, deep brownish purple with yellow streaks which run down from between the petal lobes on the outside ;

5-fid, lobes well-marked, rotate, ovate, marked by a mid-vertical line ; apex acute ; lobe-colour light purple turning to purple in old flowers, colour uniform ; orifice about 0·4 cm. in diameter. Inside of corolla tube base not hairy. Stamens five, inserted directly on throat of corolla tube ; filaments absent ; anthers small, oval-round, deep purple.

Ovary conical, green ; style about 2·7 cm. long, thin. Stigma small, bilobed.

N. Tabacum × N. plumbaginifolia, F_1. (*Plate XLVII, fig. 2*)

Habit.—Plants medium tall, about 80 cm. high, with a loose basal rosette of leaves, and a central floral axis.

Leaves.—Sessile, non-auriculate, slightly raised from ground ; lamina lanceolate ; margin wavy ; apex acute ; surface smooth with slight wrinkling along midrib towards the base ; texture medium hard ; midrib narrow at base, hairy, smooth ; hairs with terminal unicellular oval-round glands. Foliage colour green.

Inflorescence.—A central axis terminated by open cymes.

Flowers.—Medium sized, persisting for three to four days after which they are shed ; they remain open day and night. Pedicel about 1·2 cm. long, green with purple pigment towards base. Calyx loose, dilated, green with purple pigment at base and at tips of lobes ; 5-fid, lobes triangular, unequal, with acute apex ; ridges not present on calyx tube. Corolla tube medium broad, gradually dilating from about the middle ; colour brownish purple on outside, 5-fid, lobes triangular faintly marked with mid-vertical line ; often 1—3 accessory lobes are present ; occasionally more lobes are present. Colour of lobes light purple, often deeper at tips ; with age the colour becomes purple, and small white specks or stripes are often present ; occasionally stripes may be large, involving as much as three-fourths of corolla tube ; very rarely entirely white flowers are produced. Orifice about 0·8 cm. in diameter. Inside of corolla tube base densely hairy. Stamens five, epipetalous ; filaments long, fused for the greater part of the corolla tube ; distance over which fusion occurs is variable, and sometimes the whole of the filament may be fused with the corolla. Anthers medium sized, oval, purple. Pollen hyaline, shrivelled and non-functional. Ovary conical, green. Style about 4·0 cm. long, of medium thickness. Stigma medium-sized, bilobed. It will be noted that the hybrid resembles its *Tabacum* parent in respect of width of midrib at base of leaf, hairs on midrib, loose, dilated, 5-fid nerveless calyx, hairy interior of corolla base, oval anthers, and in the flowers remaining open day and night. It resembles the *plumbaginifolia* parent in respect of habit (rosette of leaves with a central floral axis), and in having non-auriculate, lanceolate leaves, coloured base and tips of calyx lobes and purple anthers.

In regard to the following characters, the hybrid is more or less intermediate :— Height of plant, margin surface and texture of leaves, foliage colour, length and colour of pedicel, size of flowers, diameter of orifice of corolla, condition of filaments, size of anthers and stigma.

The following characters are new, not being present in either of the parents :—Streaking and striping of flowers, presence of accessory corolla lobes, complete sterility. The style is longer than in either of the parents.

DISCUSSION

Perhaps the most outstanding characteristic of the hybrid is its complete sterility which probably results from chromosomal unbalance as there is a wide difference in the chromosome number of the parents. *N. Tabacum* has $n = 24$ chromosomes and *N. plumbaginifolia* has $n=10$ [East, 1928]. Back-crossing to each of the parents was tried, and also crossing with *N. rustica* L. ($n=24$), *N glutinosa* L. ($n=12$) and *N. alata* Lk. and Otto ($n=9$), without success. Endeavour was also made to obtain fertility by inducing doubling of chromosome number by exposure to ultra-violet rays* (for 2½, 5, 10 and 15 minutes), exposure to continuous light for ten days, etc. At the end of a year two of the plants died but the third was still growing vigorously and producing numerous flowers at the time of writing this note. Several branches have been successfully grafted on to *Tabacum* stocks and in this way it is hoped to continue the hybrid. Anther material of both parents and the hybrid has been preserved and it is hoped that a cytological investigation will be possible in the future.

An interesting feature is the streaking and striping of the flowers, (Fig. 1), a character not present in either of the parents. Such streaking in species hybrids in *Nicotiana* has been noted by Kostoff [1935] in distant crosses involving the species *Tabacum* and *Sanderae*. He mentions that the streaking increased with the age of the plant, an observation which is in agreement with our experience.

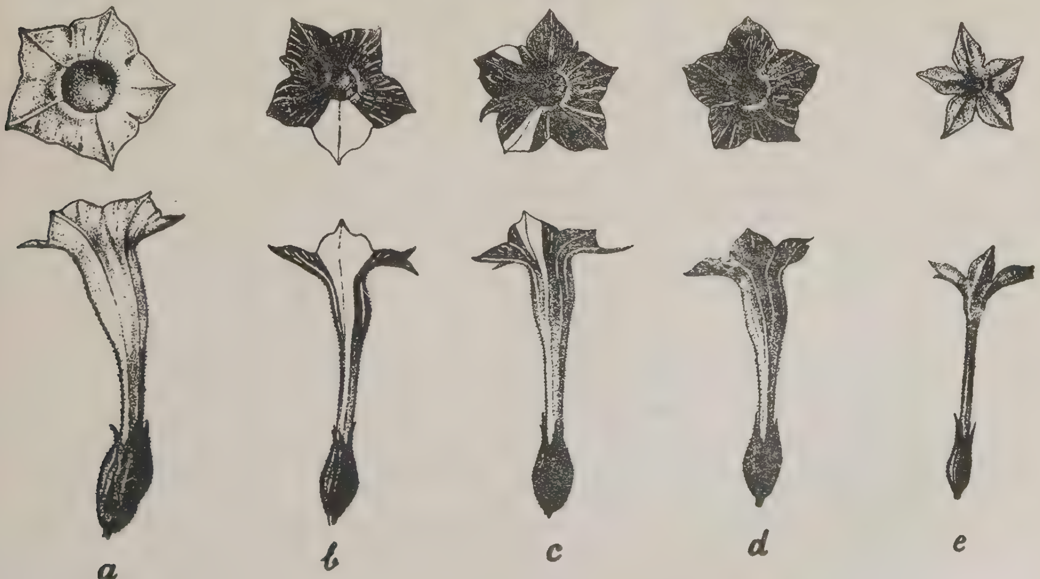

Fig. 1.

Fig. 1. Flowers of *N. Tabacum* (*a*), *N. plumbaginifolia* (*e*), and *N. Tabacum* × *N. plumbaginifolia* F_1 (*b, c, d*). (¾ nat. size).

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* This treatment was kindly carried out by Dr. B. B. Mundkur in the Mycological Section.

The greatest interest of the present cross probably lies, however, in its bearing on Goodspeed and Clausen's reaction-system hypothesis. It has already been noted by a number of investigators [East, 1928 ; Christoff, 1928, etc.] that when hybrids are obtained by crossing species differing widely in their chromosome numbers, they generally resemble the parent having the larger chromosome number. The degree of dominance of the characters of the parent with the larger chromosome number however apparently varies [East, 1928 ; Christoff, 1928 ; Malloch and Malloch, 1924 ; McCray, 1932 ; etc.]. Thus the hybrid between *N. longiflora* and *N. Tabacum* studied by Malloch and Malloch [1924] resembled more the *Tabacum* parent but showed a few *longiflora* characters. The present case is interesting in showing a condition in which the hybrid is intermediate between its parents. The *Tabacum* characters are apparently unable to " dominate " as in other crosses between *N. Tabacum* and species with low chromosome numbers.

INHERITANCE OF NICOTINE CONTENT

Determinations of nicotine content were kindly carried out by the Imperial Agricultural Chemist to whom our thanks are due. The results were as follows.

	Nicotine content (percentage)
N. Tabacum var. Pusa H. 156	1·07
N. plumbaginifolia	0·09
N. Tabacum × *N. plumbaginifolia* F_1	0·80

The hybrid is more or less intermediate, though nearer the parent with the higher nicotine content. This result may be compared with that obtained by Hackbarth and Sengbusch [1935] who found that high nicotine behaved as a simple dominant character to absence of nicotine, the F_1 showing partial dominance of the former. Kostoff and Popoff [1931] however found that in species crosses the nicotine content of the F_1's was lower than the average of the two parents, while the F_2 results indicated the presence of multiple factors.

SUMMARY

The vigorous but sterile hybrid obtained by crossing *N. Tabacum* var. Pusa H. 156 with *N. plumbaginifolia* is described.

The percentage nicotine content of the hybrid was 0·80 as compared to 1·07 of the *Tabacum* parent and 0·09 of the *plumbaginifolia* parent.

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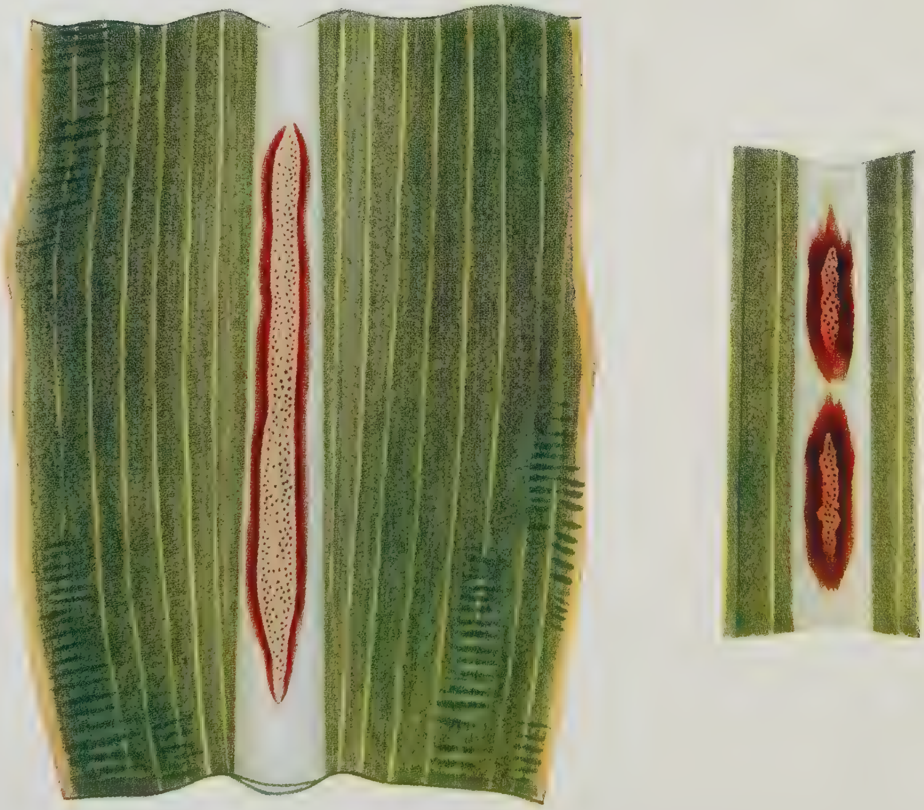

Symptoms of the disease (natural size)

A DISEASE OF *ZEA MAYS* CAUSED BY *COLLETOTRICHUM GRAMINICOLUM* (CES.) WILS.

BY

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(Received for publication on 19th February 1936)

(With Plates XLVIII and XLIX and one text-figure)

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I. INTRODUCTION

A disease of *Zea Mays* caused by *Colletotrichum* sp. was noticed at Pusa during 1934. The disease makes its appearance first on the central midrib of the leaf in the form of small brownish spots on the upper side. These spots gradually elongate along the long axis of the midrib in both directions, often reaching from the leaf-sheath to the tip of the leaf. The central portions of the spots later on become straw coloured and bear the minute black fruiting pustules of the parasite—the acervuli. Plate XLVIII shows the symptoms of the disease.

Colletotrichum cereale Manns., *C. lineola* Cda. and *C. graminicolum* (Ces.) Wils., all of which are now considered synonyms, have been reported parasitic on *Zea Mays* from North America [Seymour, 1929]. Recently a new disease of *Zea Mays* has also been reported from Russia and ascribed to *C. zeae* nov. sp. [Lobik, 1933].

II. MORPHOLOGY OF THE PARASITE ON THE HOST

Mycelium.—The mycelium of the parasite consists of slender, branched, colourless, septate haphae. These are both inter and intra-cellular and ramify in the parenchymatous tissue ; sometimes they are also observed in the vascular bundles.

Acervuli.—The acervuli (Plate XLIX, fig. 1) develop late in the season. They appear below the cuticle in stromatic masses and soon rupture the cuticle. When mature they are circular or oval, varying greatly in diameter.

Setae.—Setae appear in mature acervuli. They are mixed with the conidiophores in the acervulus and are long, dark-brown to black, thick-walled processes or bristles, somewhat pale at the tip. They are unbranched, 2 to 5-septate and measure from 4-6μ in width and up to 170μ in length. Usually the setae are broader at the base, often bulbous and gradually tapering towards the apex.

Conidiophores.—Some of the superficial cells of the stromatic mass which develops by the aggregation of fungal hyphae grow out and form the setae while the others remain short and colourless and form a crowded layer—the conidiophores—bearing the conidia at the tip. They are broader at the middle than at the ends and measure 2-7 × 1-2μ.

Conidia.—The conidia are hyaline, falcate, with a single or sometimes two oil globules and measure 18-31 × 4-5μ (Plate XLIX, fig. 1).

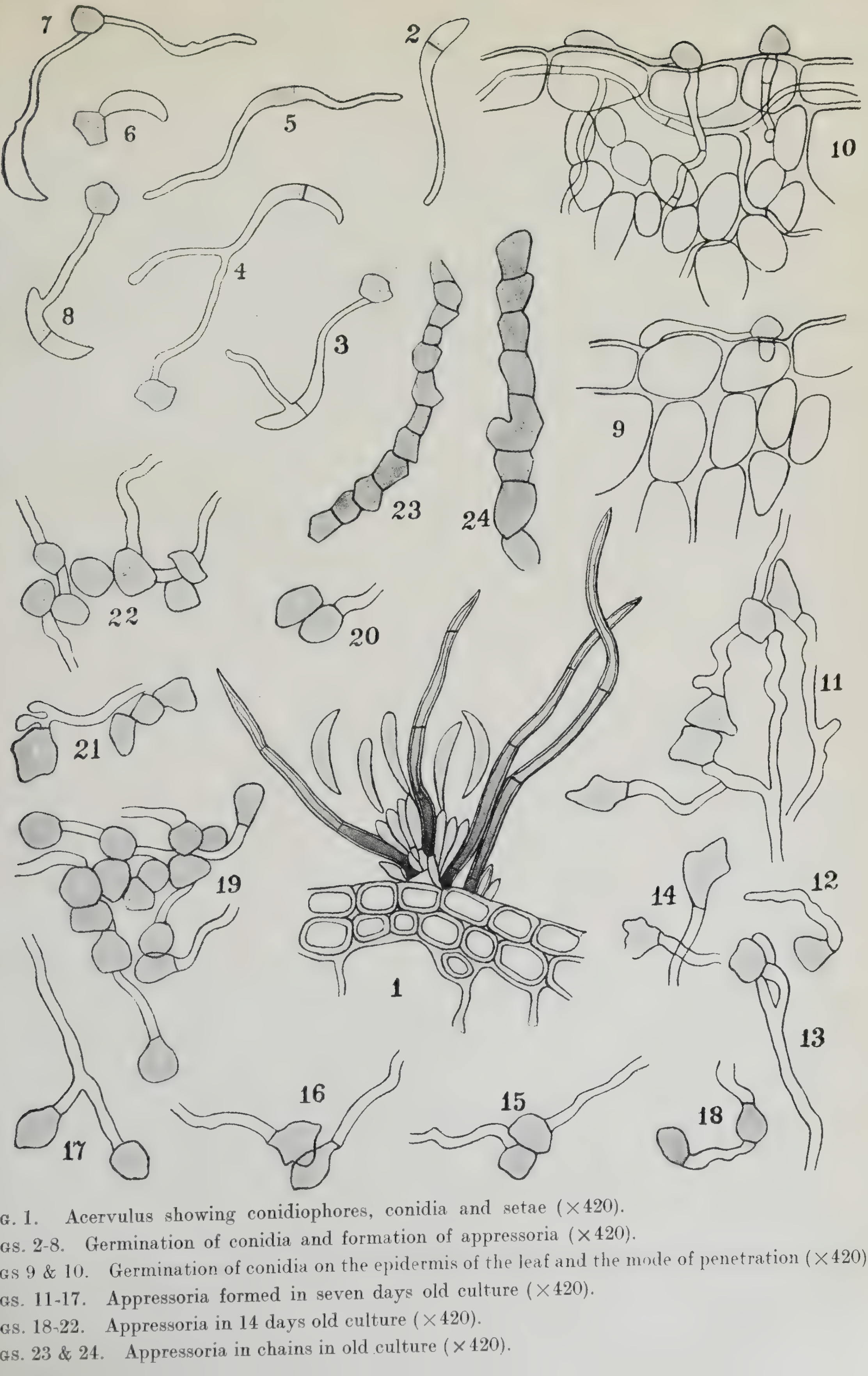
III. PARASITISM

(*i*) *Inoculation on the host.*—Single spore cultures of *Colletotrichum* sp. were obtained by the usual plating method and a series of inoculation experiments were carried out on maize plants both in the laboratory and in the field by placing spores and mycelium on unwounded host parts or by spraying with spore suspensions in sterile water. The inoculated plants were kept in moist chambers for twenty-four hours, later they were kept in the open but always kept moist by occasional spraying with water. The results of the inoculation experiments are summarised in Table I. It will appear from the table that the fungus is a parasite, capable of attacking all parts of the host plant.

TABLE I

Summary of the inoculation experiments carried out with Colletotrichum *sp. on* Zea Mays

Parts of the plant inoculated	No. of plants inoculated	No. of plants infected	No. of controls kept	No. of controls infected	Remarks
Leaves(both sides)	97	94	20	Nil	Plants kept 24 hours in moist chambers after inoculation, later in the open.
Midrib . .	89	87	32	,,	
Leaf-sheaths . .	75	70	25	,,	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3
9
23
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1
13
16
15
18
17

FIG. 1. Acervulus showing conidiophores, conidia and setae (×420).

FIGS. 2-8. Germination of conidia and formation of appressoria (×420).

FIGS 9 & 10. Germination of conidia on the epidermis of the leaf and the mode of penetration (×420).

FIGS. 11-17. Appressoria formed in seven days old culture (×420).

FIGS. 18-22. Appressoria in 14 days old culture (×420).

FIGS. 23 & 24. Appressoria in chains in old culture (×420).

(*ii*) *Mode of penetration.*—Within twenty-four hours after inoculation a great majority of the fungus spores germinate (Plate XLIX, figs. 2-8) giving out one or two germ-tubes. These germ-tubes on coming in contact with the epidermis of the host form appressoria. These appressoria in turn germinate in the same manner as the conidia (Plate XLIX, figs. 9 and 10). The appressorium becomes thinner and colourless on one side and from this spot a germ-tube appears. The germ-tube penetrates directly through the cuticle into the epidermal cell, probably by mechanical pressure as reported by Dey [1919] for *C. lindemuthianum* (Sacc. & Mag.) Br. & Cav. Stomatal entrance or penetration through wounds has not been noticed, though it is not impossible. In no case has penetration been observed except from an appressorium.

(*iii*) *Cross-inoculations.*—Numerous cross-inoculations with the fungus on a number of other related plants were made with a view to determining the possible host range by placing spores and mycelium on unwounded parts. The details of the cross-inoculation experiments are given in a tabulated form in Table II. It will appear from the table that the fungus is capable of infecting a wide range of hosts under laboratory conditions.

TABLE II

Summary of cross-inoculation experiments on various hosts with Colletotrichum *sp. from* Zea Mays

Host	Number of inoculations	Number of plants infected	Percentage of infection
Panicum typhoideum . .	35	32	91·4
Sorghum vulgare . . .	50	48	96·0
Eleusine indica . . .	48	42	87·5
Setaria italica . . .	30	25	83·3
Euchlæna mexicana . .	46	40	86·9
Panicum frumentaceum . .	49	48	97·9
Paspalum scrobiculatum . .	28	24	85·7
Hordeum vulgare . . .	37	35	94·5
Avena sativa	58	56	96·5
Triticum vulgare . . .	46	45	97·8

IV. GROWTH IN CULTURE

The fungus was grown on a large number of media. The best aerial growth with copious sporulation took place on maize-meal, oat-meal, Dox's and Hopkin's agars. Brown's synthetic agar gave a poor aerial growth and sporulation. Scanty aerial mycelium and poor sporulation were obtained on maize-leaf juice agar, maize-stem juice agar, *juar*-leaf juice agar and potato agar. Growth and sporulation were the poorest on nutrient agar. Sterilised maize stems formed a very suitable medium for copious spore formation.

In young cultures the mycelial threads are hyaline, small, septate and much branched. But gradually they darken, grow broader and become thick-walled.

In cultures three or four days old, conidia are produced at the ends of hyphae. The conidium is at first continuous with the hypha from which it is developed ; it grows in size and is then cut off from the hypha by the formation of a septum at the constricted part. The hypha then elongates and a second spore is formed. In this way several conidia are produced from a single hypha which in mass appear pinkish. Similar conidia formation in culture also occurs in other species of *Colletotrichum* [Edgerton, 1910 ; Barrus, 1921].

Acervuli are not always formed in culture. On maize and oat-meal agars they have been obtained though not in large numbers. They are similar to those formed on the host in nature. The setae are dark brown to black, light coloured at the tip, unbranched, 2-5 septate, broader (sometimes bulbous) at the base and gradually tapering towards the apex, measuring up to 170μ in length and 4-6μ in width. The conidiophores are crowded, hyaline and measure 2-7 × 1-2μ. The conidia are falcate, hyaline with one or two oil globules measuring 17-32 × 4-6μ in diameter.

Appresoria are formed in great abundance in cultures (Plate XLIX, figs. 11-22). Their number is more when the fungus sporulates poorly and does not form acervuli in culture. The appressoria are dark brown in colour and the great majority of them are simple spherical or pyriform in shape and about 8-15μ in diameter. When they are of irregular shape they may be larger. They are usually seen to develop in contact with the hard surface of the glass-tubes. In crowded and old cultures, however, they often form in the substance of the medium entirely, separated from the glass or any solid substance in the medium. Very often the appressorium has a clear white spot near the centre, the so-called " germ-pore " [Edgerton, 1910], but in some it has been found wanting. At times a large number of appressoria are seen to develop in rows (Plate XLIX, figs. 23 and 24).

V. Factors affecting sporulation

Light.—Continuous light or darkness has been found to inhibit sporulation. Cultures kept in alternate light and darkness (day and night) have been found to sporulate earlier and more copiously than those kept in continuous light or darkness.

Humidity.—Conditions of high humidity have been found to favour sporulation. When Petri dishes were grown upside down over a water surface (100 per cent relative humidity) there was more sporulation than in Petri dishes similarly held over different concentrations of sulphuric acid giving relative humidities of eighty and sixty per cent.

Sometimes sporulation has been found to be copious in cultures saturated with water. When bits of agar containing mycelium of the fungus were placed in watch glasses and saturated with distilled water copious sporulation and numerous acervuli formation took place.

Media.—Other factors being the same sporulation varied on different media. In general sporulation was more liberal when the fungus was grown on meal agars than on synthetic media. The most copious spore production, however, took place when the fungus was grown on sterilised maize stems in tubes.

TABLE III

Sporulation of Colletotrichum *sp. on different media*

Sterilised maize stems	Very good
Maize-meal agar	Good
Oat-meal agar	Good
Dox agar	Moderate
Hopkin's agar	Moderate
Brown's synthetic agar	Poor
Potato agar	Poor

Wounding.—Sporulation is accelerated in a culture by local wounding. Petri dish cultures which were wounded here and there were found to sporulate fairly strongly along the lines of wounding.

VI. SPORE GERMINATION

The time required for the germination of spores varies with the culture media used, the age of the spores, temperature, hydrogen-ion concentration, humidity, presence or absence of plant tissue.

Under the most favourable conditions spores may germinate within ten to twelve hours but they usually require somewhat longer, and some do not germinate for days, although to all appearances conditions are favourable.

Germination usually takes place by the protrusion of one or more germ-tubes (seldom more than two) from any portion of the spore (Plate XLIX, figs. 2-8). They arise most commonly from the side and near the end. The size and length of the germ-tubes differ on different media and under different environmental conditions. In nutrient media the germ-tubes commonly grow directly into profusely branching mycelium or form appressoria which in turn germinate and form a second appressorium or grows into a mycelial weft. In non-nutrient solutions appressoria are formed at the ends of the germ-tubes if conditions are favourable for their formation, otherwise the germ-tubes disintegrate without further development. A great many of the spores have been observed to become two-celled before germination.

Sugars.—Sugar solutions have been found to exert a favourable influence on the germination of spores. Spores which germinated very slowly in water, gave a higher percentage of germination when placed in sugar solutions; their germ-tubes also attained greater length.

TABLE IV

Effect of sugar solutions on the germination of spores of Colletotrichum *sp. on* Zea Mays *(based on the measurement of 100 spores)*

Media	8 hours		12 hours		24 hours		48 hours	
	Percentage germination	Average length of germ tubes μ	Percentage germination	Average length of germ tubes μ	Percentage germination	Average length of germ tubes μ	Percentage germination	Average length of germ tubes
Distilled water .	...	...	...	...	5	15	18	49
Tap water . .	2	12	5	15	19	31	42	75
Five per cent glucose solution.	8	24	25	36	78	97	96	125
Five per cent saccharose solution.	8	24	24	37	77	92	94	120

Humidity.—The effect of different relative humidities on the percentage of spore germination and the length of the germ-tubes were determined by the method suggested by Galloway [1935]. Half inch squares of thin sheet viscose (" cellophane ") which were soaked in one per cent glucose solution, and blotting off the excess of the solution, dried in air were used. Spores of the fungus were placed on these " cellophane " pieces in the form of thin streaks by folding them in half and lightly touching with the folded edge, the sporing surface of sterilised maize stems. These squares were then suspended by loops of threads in stoppered bottles of about 500 c.c. capacity, each containing 30 c.c. of sodium chloride solutions of different concentrations. These bottles were then kept in an incubator at 25°C. For each relative humidity test a large number of " cellophane " pieces were used. No " cellophane " piece was replaced in the bottle after examination. The results obtained are recorded in Table V.

TABLE V

Germination of the conidia of Colletotrichum *sp.* on Zea Mays *at different relative humidities (Data based on the measurement of 100 conidia)*

Relative humidity	16 hours		40 hours		72 hours	
	Percentage germination	Average length of germ tubes μ	Percentage germination	Average length of germ tubes μ	Percentage germination	Average length of germ tubes μ
100·0 per cent . .	56	74	96	82	...	...
96·9 " " . .	16	25	25	44	45	75
95·0 " " . .	...	..	4	20	12	48
93·9-87·5 " " . .	...	...	...	...	...	...

After seventy-two hours it was not at all possible to determine the percentage of germination and the length of the germ-tubes at 100 per cent relative humidity on account of the growth and overlapping of the germ-tubes. It will be evident, however, from results recorded in Table V that the best germination of the conidia takes place at 100 per cent relative humidity. At lower humidities there is a gradual fall in the rate of germination and no germination takes place below ninety-five per cent relative humidity.

Temperature.—To determine the effect of temperature on the germination of conidia, loops of spore suspension in sterile water were placed on clean cover slips which were then inverted over glass rings cemented to slides. A small quantity of sterile water was placed at the bottom of each cell. These hanging drops—four of each—were then placed in incubators running at different temperatures. The percentage of germination and the average length of germ-tubes were calculated from a count of 100 conidia at random in each cell. The results obtained are recorded in Table VI.

TABLE VI

Effect of temperature on the germination of conidia of Colletotrichum *sp. on* Zea Mays

Temperatures	16 hours		48 hours		Remarks
	Percentage germination	Average length of germ-tubes μ	Percentage germination	Average length of germ-tubes μ	
20°C. . . .	24	18	38	35	Germ-tubes normal
25°C. . . .	36	32	65	56	,, ,, ,,
30°C. . . .	52	46	88	75	Germ-tubes abnormal.
32·5°C. . .	63	52	92	79	,, ,, ,,
35°C. . . .	..	..	..	..	

It will be noticed from the table that the optimum temperature for the germination of conidia lies between 25°C. and 30°C.

Plant tissue.—Several investigators have found that the presence of host tissue accelerates the germination of spores of various pathogens. Anderson [1914] showed it for pycnospores of *Endothia parasitica* (Mur.) Anders.; Whitehead [1920] for spores of *Urocystis cepulae* Frost.; Brown [1922] for conidia of *Botrytis cinerea* Pers.; Leach [1923] for spores of *Colletotrichum lindemuthianum* (Sacc. and Mag.) Br. and Cav.; Noble [1924] for spores of *Urocystis tritici* Koern; Christensen [1926] for spores of *Helminthosporium sativum* P. K. and B.; and Mitra and Mehta [1934] for spores of *H. nodulosum* B. et C. Besides, the experiments of Christensen [1922], Leach [1923], Noble [1924] and Mitra and Mehta [1934] show that tissues of plants other than the host also stimulate the germination of spores.

The effect of plant tissues on the germination of conidia of the fungus were studied by placing conidia in watch glasses containing from 0·5 c.c. to one c.c. of distilled water. To these watch glasses from ten to twenty small pieces of plant tissues were added. These plant tissues were first carefully washed with 1 : 1000 solution of mercuric chloride and then the last traces of mercuric chloride removed by several washings with sterile distilled water. The controls contained distilled water only. All tests were made in duplicate and at room temperature (about 30°C.) The percentage of germination and the average length of germ-tubes were calculated from a count of 100 spores at random in each dish. The results obtained are recorded in Table VII.

TABLE VII

Effect of plant tissue on the germination of conidia of Colletotrichum *sp. on* Zea Mays

Plant tissue	20 hours	
	Percentage germination	Average length of germ-tubes(μ)
Control	12	60
Zea Mays (boiled)	12	60
Saccharum officinarum	21	87
Brassica campestris	28	109
Glycine hispida	35	129
Eleusine indica	42	131
Triticum vulgare	68	168
Sorghum vulgare	70	168
Panicum frumentaceum	70	167
Zea Mays	72	172

It will be observed from Table VII that the best germination took place in the watch glasses containing the host tissues. Presence of un-related plant tissues also enhanced the germination of spores. In the presence of *Sorghum vulgare, Triticum vulgare* and *Panicum frumentaceum* tissues the condia germinated almost as well as in the presence of *Zea Mays* tissue.

In the control it was noticed that there was a higher percentage of germination of conidia floating on the surface than those submerged in water. On the contrary little or no difference could be observed in the percentage of germination of floating or submerged conidia, in watch glasses containing plant tissues. Christensen [1926] and Mitra and Mehta [1934] have also made similar observations for conidia of *Helminthosporium sativum* P. K. and B. and *H. nodulosum* B. et C. respectively. Brown [1922] found that certain plants produce volatile substances which either stimulate or inhibit spore germination. Christensen [1922] working on *Helminthosporium sativum* concludes " apparently the stimulatory agent is not specific." It was found, however, that boiled leaves of maize were incapable of stimulating germination; this shows that the stimulatory principle is destroyed by heat. Germ-tubes were found always directed towards plant tissues showing that the plant tissues exerted some "chemotrophic influence" [Mitra and Mehta, 1934].

VII. COMPARISON OF *COLLETOTRICHUM* SP. ON *ZEA MAYS* WITH *C. GRAMINICOLUM* (CES.) WILLS. ON *SORGHUM VULGARE*

(*i*) *Inoculation experiments.*—With a view to determine whether *Colletotrichum* from *Zea Mays* can infect *Sorghum vulgare* and *vice versa*, a large number of cross-inoculation experiments were carried out. The results of the inoculation experiments are tabulated in Table VIII.

TABLE VIII

Summary of inoculation experiments carried out with Colletotrichum *sp. from* Zea Mays *and* C. graminicolum *on* Sorghum vulgare *and* Zea Mays *respectively*

Fungus	Plants inoculated	Number of inoculations	Number of infection	Percentage of infection	Remarks
Colletotrichum sp. from *Zea Mays*	*Sorghum vulgare*	36	36	100	Controls were kept and were intact.
C. graminicolum.	*Zea Mays*	32	32	100	

It will be noted from the above table that the fungus isolated from *Zea Mays* is capable of infecting *Sorghum vulgare* and that *C. graminicolum* isolated from *Sorghum vulgare* is capable of infecting *Zea Mays*. In both the cases all the plants inoculated took infection while the controls remained intact.

(*ii*) *Morphological characters.*—In shape and size the conidia of *Colletotrichum* sp. occurring naturally on *Zea Mays* agree very closely with those of *C. graminicolum* on *Sorghum vulgare*. A measurement of two hundred conidia, each from *Zea Mays* and *Sorghum vulgare* showed that the range of conidia measurements was 18-31 × 4-5μ for *Colletotrichum* sp. on *Zea Mays* and 19-30 × 4-5μ for *C. graminicolum* on *Sorghum vulgare*. The means for lengths and breadths with their standard errors were $24 \cdot 90 \pm 0 \cdot 2261 \times 4 \cdot 502 \pm 0 \cdot 0352$ for *Colletotrichum* sp. on *Zea Mays* and $24 \cdot 70 \pm 0 \cdot 2050 \times 4 \cdot 55 \pm 0 \cdot 0351$ for *C. graminicolum* on *Sorghum vulgare*. It is clear, therefore, that there exists no significant difference between the two fungi from the morphological point of view.

(*iii*) *Cultural characters.*—*Colletotrichum* sp. from *Zea Mays* and *C. graminicolum* from *Sorghum vulgare* were grown on maize-meal agar, oat-meal agar, Dox's agar, Brown's synthetic medium, Hopkin's medium and Coon's agar under identical conditions for comparison.

Colletotrichum sp. from *Zea Mays* resembles *C. graminicolum* in the following :—

(*a*) Production of white cottony or fluffy type of mycelial growth and in the formation of acervuli and appressoria in culture.

(*h*) Similar growth rate at different temperatures and on a variety of media. Fig. 1 which gives the growth rate after seven days on Hopkin's, Brown's and Dox's media show how closely they resemble.

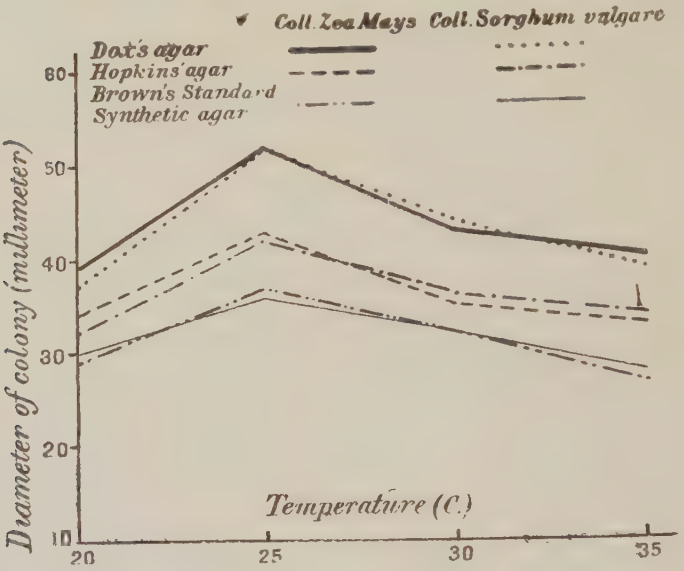

Fig 1. Comparative growth rate of *Colletotrichum* sp. from *Zea Mays* and *Sorghum vulgare* on various media.

Colletotrichum sp. on *Zea Mays* agrees closely with *C. graminicolum* as regards the dimension of spores on different media. Under the same cultural conditions the range of spore length was very close. Two hundred spores were measured at random from oat-meal cultures fifteen days old and grown under the same environmental conditions. The range of spore length and width for *Colletotrichum* from *Zea Mays* was 17-32 × 4-6μ and for *Sorghum vulgar Colletotrichum* 19-31 ×4-6μ.

VIII. CONCLUSION AND SUMMARY

A disease of *Zea Mays* caused by a species of *Colletotrichum* was observed at Pusa. The fungus was isolated and its morphology, parasitism, cultural characters together with the effect of environmental factors on sporulation and on the germination of spores were studied.

Inoculation experiments carried out have shown that the organism can attack all the parts of the plant—leaves, midribs and leaf-sheaths. Cross-inoculation experiments carried out on a large number of allied plants showed that the parasite has a wide range of hosts under laboratory conditions.

Studies of the parasite in culture reveal that it grows better on natural than on synthetic media. Sporulation is favoured by high humidity and by alternate light and darkness. Acervuli with setae are not frequently formed in culture. When the fungus sporulates poorly, appressoria are formed in great abundance either in contact with the hard surface of the glass container or in the substance of the medium.

It was noticed that high humidity, the presence of sugar, or the presence of living plant tissues accelerate the germination of spores. The optimum temperature for the germination of spores was found to be between 25°C and 30°C.

The fungus was compared with *C. graminicolum* isolated from *Sorghum vulgare*. The *Zea Mays* fungus can infect *Sorghum vulgare* and *vice versa*. The cultural characters and spore measurements show that both are very closely allied and thus the conclusion is drawn that the *Colletotrichum* sp. from *Zea Mays* is identical with *C. graminicolum* (Ces.) Wils.

ACKNOWLEDGMENTS

My grateful thanks are due to Mr. L. D. Galloway, Imperial Mycologist, for facilities offered and keen interest taken in this work. I am also greatly indebted to Dr. M. Mitra for the suggestion of the problem and the ungrudging guidance throughout the work and in the preparation of the manuscript.

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REDUCTION OF NITRATES BY THE SUN

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(Received for publication on 8th January 1936)

Berthelot and Gaudechon [1911] found that ultra-violet light induced the reduction of potassium nitrate in solution. The reduction was more in alkaline than in neutral or acidic solutions. Further in very weak solutions the reduction was practically complete.

Corbet [1934] using sunlight instead of ultra-violet light found that sunlight had a similar action though in a lesser degree.

The percentage of ultra-violet radiations in a beam of sunlight is small, yet it is an important source of ultra-violet light especially in tropical countries where the intensity of the sun is great. Therefore preliminary studies on the reduction of nitrates and the conditions affecting it were undertaken.

Experimental.—Fifty c.c. of potassium nitrate solution (one mg. nitrogen per c.c.) was taken in each of the twelve Pyrex conical flasks of 300 c.c. capacity. Six of these were wrapped in black paper. These along with the rest were exposed to the sun. Estimations of nitrous nitrogen were made by the usual Griess Illosvay calorimetric method. The reduction is shown in Table I.

TABLE I

Showing the reduction of potassium nitrate

Time (days)	Mg. nitrous nitrogen per 100 c.c.	
	Unexposed	Exposed
1	*Nil*	0·039
	,,	0·036
2	,,	0·068
	,,	0·064
3	,,	0·091
	,,	0·091

For comparison the solution of the same strength was exposed to ultra-violet light from mercury arc lamp consuming about 1·6 amp. The solution was put in open Petri dishes and was placed directly one foot below the burner. There was considerable rise of temperature during the exposure. Representative samples for analysis were withdrawn after ten, twenty, thirty and forty minutes.

TABLE II

Showing the effect of ultra-violet radiations on the reduction of potassium nitrate

No.	Exposure (minutes)	Mg. nitrous nitrogen per 100 c.c.	Mean
1	10	0·110 0·123 0·104	0·112
2	20	0·220 0·233 0·229	0·227
3	30	0·311 0·362 0·337	0·337
4	40	0·427 0·518 0·440	0·461

The results given in Table II showed that ultra-violet light was a powerful reducing agent as compared to the sunlight.

Since ultra-violet light is absorbed by glass, the reduction of the substrate contained in Pyrex glass flasks and open Petri dishes of the same diameter was studied. Fifty c.c. of potassium nitrate solution containing one mg. nitrogen per c.c. was put in each of the containers and exposed to the sun. Loss of moisture was made up every half an hour. The contents were analysed after eight hours

TABLE III

Showing the reduction of potassium nitrate in flasks and Petri dishes

Container	Mg. nitrous nitrogen per c.c.	Average
Flasks	0·039 0·042 0·039 0·032	0·038
Petri dishes	0·045 0·064 0·045 0·054	0·058

The results (Table III) showed that the efficiency of the solar radiations was reduced by about 30 per cent by passage through Pyrex glass.

The nitrates of calcium, sodium, potassium, ammonium, magnesium aluminium, manganese and iron were similarly reduced when exposed to the sun.

The reduction of potassium nitrate was further studied with regard to the following :—

1. Concentration.
2. Temperature.
3. Season.
4. Influence of other substances.

1. Effect of concentration

Potassium nitrate solution (in 50 c.c. lots) of different concentrations was exposed to the sun in 300 c.c. conical flasks for forty-eight hours.

TABLE IV

Showing the effect of concentration on the reduction of potassium nitrate

Serial No.	Mgs. nitrogen per 100 c.c.	Mg. nitrous nitrogen	Percentage reduced
1	1·0	0·007	0·7
2	2·0	0·013	0·65
3	5·0	0·024	0·48
4	10·0	0·032	0·32
5	20·0	0·048	0·24
6	50·0	0·088	0·18
7	100·0	0·169	0·17

It is evident from Table IV that the percentage of reduction decreases with increasing concentration.

2. Influence of temperature

Three different temperatures, *viz.*, 10°, 25° and 45°C. were tried. Hundred c.c. of potassium nitrate solution (one mg. nitrogen per c.c.) was taken in each of the six 300 c.c. conical flasks. These were placed in duplicates in three white enamelled trays containing water at different temperatures and exposed to the sun. Five-c.c. samples were withdrawn at ten minutes interval and treated with 0·5 c.c. of the reagent, put in $\frac{1}{2}$-in. cell and compared with standard slides.

TABLE V

Showing the effect of temperature on the reduction of potassium nitrate

Exposure (minutes)	10°C.±1·5	25°C±0·5	45°C.±1·5
10	1·1	1·4	1·6
20	1·6	1·9	2·2
30	2·0	2·2	2·6
40	2·2	2·5	3·3
50	2·4	3·1	3·5
60	2·6	3·3	4·0

The result given in Table V showed that the reduction of potassium nitrate increased with increasing temperature.

3. Effect of season

The reduction of potassium nitrate solution (one mg. nitrogen per c.c.) in fifty c.c. portions was studied during different months.

TABLE VI

Period of exposure (days	Mg. nitrous nitrogen per 100 c.c.		
	January	May	September
1	0·016 0·015	0·045 0·045	0·039 0·036
2	0·024 0·023	0·089 0·065	0·068 0·064
3	0·032 0·029	....	0·091 0·091

The results (Table VI) showed that the reduction during summer was about thrice as much as in winter.

4 (a) Reduction of potassium nitrate in the presence of reduced nickel

Fifty c.c. of potassium nitrate solution (one mg. nitrogen per c.c.) was taken in six 300 c.c. conical flasks. Reduced nickel (0·5 grm.) was added to four of them. All the flasks were placed in the sun; two of them with nickel being wrapped in black paper.

TABLE VII

Treatment	Condition	Mg. nitrous nitrogen per 100 c.c. solution after			Percentage reduced
		1 day	2 days	8 days	
With nickel .	Unexposed	*Nil*	*Nil*	*Nil*	..
”		”	”	”	
Without nickel .	Exposed .	0·036	0·068	0·356	0·332
	”	0·032	0·062	0·308	
With nickel .	”	0·071	0·156	0 972	0·988
”	”	0·068	0·169	1·004	

The results in Table V showed that the reduction was more in the presence of reduced nickel, than without it.

4 (*b*) *Reduction of potassium nitrate in the presence of carbohydrates*

Fifty c.c. of potassium nitrate solution (one mg. nitrogen per c.c.) was taken in each of twenty-four conical flasks of 300 c.c. capacity. These were divided into six sets and each set was treated as shown below :—

1. No treatment.
2. 0·5 grm. sucrose per flask.
3. 0·5 grm. fructose per flask.
4. 0·5 grm. dextrose per flask.
5. 0·5 grm. maltose per flask.
6. 0·5 grm. potato starch per flask.

The flasks were plugged with cotton wool. Two flasks in each set were wrapped in black paper, and these along with the rest were kept in the sun. The contents of the flasks were analysed after an exposure of seven hours.

TABLE VIII

Showing the reduction of potassium nitrate in the presence of carbohydrates

Serial No.	Carbohydrates	Mg. nitrous nitrogen per 100 c.c.
1	Control	0·032 0·030
2	Sucrose	0·075 0·075
3	Fructose	0·075 0·071
4	Dextrose	0·078 0·084
5	Maltose	0·068 0·068
6	Starch	0·052 0·052

The results (Table VIII) showed that the reduction was more in the presence of carbohydrates. Starch gave the lowest figures, while it was practically the same in the case of other carbohydrates. Nitrous nitrogen in the flasks wrapped in black paper was only in traces.

The reaction is obviously a photo-chemical one, as the reduction in the dark was negligible. It is possible that the action of these substances may be a catalytic one.

4 (c) *Influence of zinc oxide*

Since zinc oxide is a powerful oxidising catalyst, it was interesting to see its effect on the reduction of potassium nitrate. It was found out that the reduction was completely stopped in its presence.

4 (d) *Reduction of nitrates in the presence of soil*

Soil has been found to act as a photo-catalyst for the oxidation of nitrogenous compounds [Sarkaria and Fazal-ud-Din, 1933]. The reduction of nitrates in its presence was also studied.

Fifty c.c. of potassium nitrate solution (one mg. nitrogen per c.c.) was put in each of the eight conical flasks of 300 c.c. capacity. Five grms. of soil were put in each flask. These were plugged with cotton wool and sterilized. Paper was tied over the plugs to protect them from dust. Four flasks were covered with black paper. These along with the rest were kept in the sun. A similar series with another soil was set up side by side. The contents of the flasks were analysed after twenty-five days.

TABLE IX

Showing the reduction of nitrate solution in presence of soil

Soil taken	Mg. nitrous nitrogen per 100 c.c.		Percentage reduced
	Exposed	Unexposed	
Loam	0·745	0·005	0·68
	0·606	0·006	
	0·713	0·007	
	0·680	0·006	
Clay loam	0·606	0·011	
	0·745	0·012	0·69
	0·713	0·012	
	0·745	0·013	

The results given in Table IX showed that nitrates were reduced in the presence of soil.

In order to find out the influence of soil on nitrate reduction, the experiment described above was re-started with varying amounts of soil (five and twenty grams) and four control flasks without soil. The contents of the flasks were analysed after twenty-five days.

TABLE X

Showing the effect of soil on the reduction of potassium nitrate in solution

(Mg. nitrous nitrogen per 100 c.c.)

Serial No.	Without soil	Loam		Clay loam	
		5·0 grms.	20·0 grms.	5·0 grms.	20·0 grms.
1	0·713	0·743	0·741	0·603	0·639
2	0·713	0·604	0·708	0·742	0·769
3	0·713	0·711	0·741	0·710	0·597
4	0·648	0·678	0·601	0·742	0·769
Average	0·697	0·684	0·698	0·699	0·693

The results (Table X) showed that the soils had no influence on reduction. It seems that the catalytic action of the soil is very feeble. Although, it is an oxidising catalyst, reduction took place in its presence without being influenced in any way by it, while in the presence of zinc oxide, potassium nitrate could not be reduced by the sun. It is obvious that the rate of photo-chemical reduction in the presence of soil is considerably greater than the rate of oxidation of nitrite to nitrate.

REDUCTION OF NITRATES IN SOIL

Two soils were moistened with potassium nitrate solution just below the sticky point. The nitrate solution in each case was of such a strength as to supply 100 mgs. of nitrogen per 100 grms. of soil. Fifty grams soil (on dry basis) was put in each of the eight 300 c.c. conical flasks. These were plugged with cotton wool and sterilized. Paper was tied over plugs to protect them from dust. Four flasks of each soil were wrapped in black paper and these along with the rest were exposed to the sun. Loss of moisture was not made up. The soils looked quite dry after a week. Nitrous nitrogen was estimated after twenty-five days.

TABLE XI

Showing the reduction of nitrates in soil

Soil taken	Mg. nitrous nitrogen per 100 c.c.		Percentage reduced
	Exposed	Unexposed	
Loam	0·047 0·054 0·039 0·050	0·021 0·027 0·019 0·019	0·026
Clay Loam	0·047 0·054 0·039 0·050	0·023 0·023 0·021 0·027	0·024

The results given in Table XI showed that nitrates were appreciably reduced in soil.

Nitrite formation in the exposed flasks may either be attributed to the oxidation of nitrogenous compounds of the soil or to the reduction of nitrates. But on exposing soils without potassium nitrate under identical conditions, no nitrite was obtained, therefore the excess of nitrite in the exposed flasks was due to the reducing action of the sun.

The results of reduction in soils, when treated statistically, gave the value of *S*, *S.E.* and '*t*' to be 0·00479, 0·00239 and 10·8 for loam soil and 0·00558, 0·00279 and 8·5 for clay loam soil. The expected value of '*t*' at one per cent from Fisher's table is only 5·487. Hence the results are significant.

The experiment was repeated with a smaller dose of potassium nitrate (ten mgs. nitrogen per 100 grms. of soil) and the results confirmed.

The above experiments seem to indicate that photo-reduction of nitrates in well-drained soils is of no importance. In case of water-logging the nitrates may partly be reduced photo-chemically.

EFFECT OF MOISTURE

Ordinary field soil was supplied with 100 mgs. of nitrogen as potassium nitrate per 100 grms. of air-dry soil. It was distributed in fifty-gram portions in twelve 300 c.c. conical flasks.

The soil in four flasks was kept in air-dry state. Out of the rest eight flasks, four were kept at thirteen per cent and four at thirty-nine per cent moisture. The flasks were closed with rubber bungs and sterilized. Analysis was done after eight days' exposure.

TABLE XII

Showing nitrate reduction in soil under different moisture conditions

Serial No.	Mg. nitrous nitrogen per 100 grms. soil		
	Air-dry	13 per cent moisture	39 per cent moisture
1	0·044	0·073	0·073
2	0·044	0·074	0·083
3	0·044	0·068	0·068
4	0·044	0·073	0·074
Average .	0·044	0·072	0·074

Initial nitrous nitrogen 0·039 mg.

The results given in Table XII showed that in dry soil the reduction was negligible. At thirteen per cent to thirty-nine per cent moisture it was appreciable and was un-influenced by moisture contents.

In order to see whether the effect of moisture was significant or not, the value of '*t*' was calculated. The results are highly significant as the calculated value of '*t*' is 21·7, while the value of '*t*' at one per cent from Fisher's tables is only 5·487.

EFFECT OF FARMYARD MANURE

Two per cent fermented cow dung was added to a portion of the soil and was thoroughly mixed. Five-gram portions of the manure and unmanured soil were added separately in flasks, containing fifty c.c. of potassium nitrate solution (one mg. nitrogen per c.c.) and sterilized. Four flasks, two of each lot were wrapped in black paper and these along with the rest were exposed to the sun and were analysed after twenty-two days' exposure.

TABLE XIII

Showing the effect of farmyard manure on the reduction of nitrates in solution

Soil treatment	Mg. nitrous nitrogen per 100 c. c.		Difference
	Exposed	Unexposed	
Manured	0·713 0·680	0·018 0·018	0·678
Unmanured	0·583 0·583	0.008 0.008	0·575

The results given in Table XIII showed that farmyard manure slightly increased reduction in nitrate solution.

The control soil flasks with fifty c.c. of distilled water were also exposed side by side with the other flasks to see if there was any nitrite production due to the oxidation of the organic matter, which was found to be nil.

The reduction was also tried in manured and unmanured soil, and was found to be practically the same in both.

EFFECT OF SOIL PACKING

A soil with thirteen per cent moisture and 100 mg. nitrogen as potassium nitrate per 100 grms. of air-dry soil was distributed in eight conical flasks in fifty-gram portions on air-dry basis. The soil in four flasks was well pressed with a glass rod with a flattened end, while in the other flasks it was simply tapped to level the surface. The flasks were closed with rubber bungs and exposed to the sun after sterilization. The analysis was done after eight days.

TABLE XIV

Showing the effect of packing on the reduction in soil

Serial No.	Mg. nitrous nitrogen per 100 grms. soil		Mean difference
	Loose	Packed	
1	0·087	0·068	0·017
2	0·087	0·068	
3	0·078	0·068	
4	0·078	0·058	

The results given in Table XIV showed that the reduction was slightly more in loose packed than in hard-packed soil.

The penetration of light and surface area were less in compact soil than in the loose packed soil, hence the reduction was less in compact than in loose soil. The calculated value of '*t*' in this case is 4·89 while the expected value of '*t*' at two per cent from Fisher's table is 4·54. Hence the results are significant.

CONCLUSIONS

1. Nitrates of sodium, potassium, calcium, magnesium, manganese, ammonium, aluminium and iron were all reduced by the sun.
2. The lower the concentration, the higher was the percentage of reduction.
3. The nitrate reduction increased with the rise in temperature.
4. Reduced nickel, sucrose, fructose, dextrose, maltose and starch helped nitrate reduction.

5. The reduction of potassium nitrate in solution was not influenced in any way by the presence of soil.

6. The reduction was uniform within a wide range of moisture.

7. The reduction of nitrates in manured and unmanured soil was found to be practically the same.

8. The reduction was slightly more in a loose packed soil than in hard packed soil.

9. Photo-reduction of nitrates does not seem to be of any importance in soils.

ACKNOWLEDGMENTS

The writer wishes to thank Dr. S. V. Desai, the Agricultural Bacteriologist, and S. Ram Singh Sarkaria for helpful suggestions during the course of the work.

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INHERITANCE OF SOME MORPHOLOGICAL CHARACTERS IN *CREPIS DIOSCORIDIS**

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(Received for publication on 17th January 1936)

Introduction

Since 1920 when Babcock pointed out the advantages of the genus *Crepis*, comprising about 200 species, for genetic investigations, considerable work has been done by members of the Division of Genetics of the University of California. Perhaps the most outstanding work on the inheritance of morphological characters in Crepis has been done by Rau [1923] and Collins [1924]. Rau [1923] reported investigations on the inheritance of three quantitative characters in *Crepis capillaris* (L.) Walb., *viz.*, length of leaves, number of lobes per leaf, and diameter of flower heads. He found that the length of leaf and the number of lobes per leaf, are heritable characters, but whereas environment greatly influences the expression of the former, it has little influence on the latter. At least four pairs of genes seem to be involved in conditioning the number of lobes per leaf. He selected two races of *Crepis capillaris* with different diameters of capitula and on crossing found that the diameter of the capitula of F_1 was intermediate between those of the two parents; no F_2 data were reported. He found moisture to be the limiting factor in determining the flower size.

Collins [1924] reported on the inheritance in *C. capillaris* of nineteen morphological and three physiological characters. The same author working with *Crepis dioscoridis* in 1926 found a plant which was wholly devoid of anthocyanin, and when crossed with the common pigmented type, the F_1 was fully pigmented. The F_2 cultures, segregated as follows : 103 purple to 38 green plants, clearly a simple 3 : 1 ratio. He concluded that one main gene conditions the production of any pigment in *C. dioscoridis*. Haney [1930] noted that yellowish green foliage colour in *C. dioscoridis* behaved as a simple recessive to normal green but no data was presented.

The present investigation deals with the inheritance of the following pairs of characters : presence or absence of anthocyanin, green or yellow foliage colour and normal or bushy habit of growth. The work was carried on partly in the greenhouse and partly in the field and the data combined.

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*The investigation herein reported is a part of the thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Genetics in the Graduate Division of the University of California.

MATERIAL AND METHODS

Seeds were germinated in sterile sand in two-inch earthen pots. The seeds were lightly covered and the pots placed in a shallow pan nearly full of water. Thus the sand absorbed enough water for germination of seeds. The majority of the seedlings emerged after four days and were planted singly in four-inch pots. Notes regarding the colour of the plants, whether green or yellow, were taken at the time of transplanting. The plants homozygous for the "yellow" gene may be recognized shortly after the seedlings appear, usually one day after, but notes were not taken until transplanting time so that the full quantity of chlorophyll peculiar to the respective individuals might develop.

Then, after about a month the plants were transplanted into six-inch pots. After another month the plants were set in the field where they could have the full sun. Normal *C. dioscoridis* is quite heavily pigmented with anthocyanin but in some cases the pigmentation is quite dilute and un-uniformly distributed. So, in determining the pigmented and non-pigmented types, plants should be grown in full light. Under such conditions pigment will be developed uniformly in the basal portion of flower stalks, except in those plants homozygous for the recessive condition. The two types can usually be distinguished in the early seedling stage if the seedlings are exposed to light and moderately low temperature. In such cases, the hypocotyl becomes pink in plants capable of producing anthocyanin and remains white or green in those lacking this ability. However, to avoid possible mistakes in classification of the two types, notes were completed when the plants had been about a month in the field.

Plants from culture 29·108 were intended for the determination of habit of growth whether normal or bushy and were, therefore, not set out in the field, because condition of the soil in the field may be very heterogeneous and uniformity in the environmental conditions is most desirable for the accurate determination of this character. So the plants were left in the greenhouse in the six-inch pots with an equal amount of potting soil in each pot.

In presenting data from hybrid populations, the degree of correspondence of observed with calculated distributions has been determined by the use of tables of probable error of Mendelian ratios prepared by the Department of Plant Breeding of Cornell University. In testing goodness of fit of dihybrid ratios, the method suggested by Harris [1912] has been used.

Brief description of cultures used

F_1 Culture—28·25= (26·1631-38 × 26·1740-69).

26·1631-38 Tall, anthocyanin absent, hairy, marginal achenes white, herbage green.

26·1740-69 Tall, anthocyanin present, glabrous, marginal achenes white, herbage yellow.

Six plants were isolated in this culture and all were tall, with anthocyanin present, and herbage normal green.

Plant 1—glabrous, marginal achenes dark or slightly whitened on ventral side.
Plant 2—hairy, marginal achenes white.
Plant 3—glabrous, marginal achenes white.
Plant 4—glabrous, marginal achenes dark or slightly whitened on ventral side.
Plant 5—hairy, marginal achenes white (occasionally one dark).
Plant 6—glabrous, marginal achenes dark or slightly whitened on ventral side.

These plants were grown together so that they may have inter-crossed among themselves.

F_1 Culture—29· 108=(26·1455-3 × 26·1742-5)

26·1455-3 Tall, anthocyanin present, hairy, marginal achenes white, herbage normal green.

26·1742-5 Bushy, anthocyanin present, hairy, marginal achenes white, herbage normal green.

Seven plants were isolated from culture 29.108. All were tall, with anthocyanin present and hairy. Plant 6 was the only one with dark marginal achenes. For growing the F_2 population a small portion of seed from the progeny of each plant was used.

EXPERIMENTAL RESULTS

Inheritance of anthocyanin

Anthocyanin is found in many parts of the plant but is most noticeable in the midribs of the leaves and on the lower portions of the stems. The degree of its development may vary with different plants. In the plants with anthocyanin present the ligules usually have a red stripe on the back. Table I shows the data on segregation of hybrid plants.

TABLE I

Monohybrid segregation of non-pigmented plants from the progeny of pigmented plants

Culture 28·25	Total No. of plants	No. of plants with anthocyanin	No. of plants with no anthocyanin	Calculated		Deviations
Plant 1 . . .	31	26	7	24·75	8·25	1·25±1·63
Plant 2 . . .	83	63	20	62·25	20·75	0·75±2·66
Plant 3 . . .	66	53	13	49·50	16·5	3·50±2·37
Plant 4 . . .	95	72	23	71·25	23·78	0·75±2·85
Plant 5 . . .	62	46	16	46·50	15·5	0·50±2·30
Plant 6 . . .	98	77	21	73·50	24·5	3·50±2·89
Total .	435	337	98	326·25	108·75	10·75±6·09

The ratio in this case is 337 pigmented (red) to 98 non-pigmented (not red) plants and the deviation is less than twice the probable error. This is clearly a uni-factorial ratio, therefore this character is conditioned by a single dominant gene difference.

That this factor pair (**Aa**) controlling the production of anthocyanin is separately inherited from (**Gg**) a factor pair for chlorotic foliage colour or " yellow " is indicated by the F_2 distribution shown in Table II.

TABLE II

Dihybrid segregation in the progeny of self-fertilized F_1 plants (**AaGg**) *of culture 28·25*

	Total No. of plants	No. of red-green (**A-G**) plants	No. of red-yellow (**A-g**) plants	No. of non-red green (**a-G**) plants	No. of non-red yellow (**a-g**) plants	Deviation
Observed .	431	252	83	75	21	$\chi^2 = 4{\cdot}48$
Calculated 9 : 3 : 3 : 1	..	242·4375	70·8125	70·8125	26.9375	($P = 0{\cdot}2174$)

The goodness of fit value in this case is about twenty-one per cent which clearly indicates that these two factors are independently inherited. By using Immer's method for calculating linkage intensities [Immer, 1930] the ratio of product value is 0·8501 and the crossing-over value about forty-eight per cent which also indicates no linkage between these two factors.

Inheritance of " yellow "

" Yellow " (**gg**) is a recessive condition characterized by dilution of the quantity of chlorophyll and tentatively called " yellow " from the yellowish green colour as compared with the dark full-green pigmentation normal to the species. " Yellow " plants are usually a little smaller, grow more slowly, and mature later than the normals. Individual plants vary in degree of yellow colour, that is, some are deeper yellow than the others. It is quite possible that these differences may be merely due to the differences in nutrition.

The data are presented in Table III.

TABLE III

Monohybrid segregation of " yellow " plants from the progeny of F_1 green plants

Culture 28·25	Total No. of plants	No. of green plants	No. of yellow plants	Calculated 3	Calculated : 1	Deviation from 3 : 1
Plant 1 .	32	25	7	24·00	8·00	1·00 ± 1·65
Plant 2 .	85	62	23	63·75	21·25	1·75 ± 2·69
Plant 3 .	69	52	17	51·75	17·25	0·25 ± 2·43
Plant 4 .	100	74	26	75·00	25·00	1·00 ± 2·92
Plant 5 .	64	50	14	48·00	16·00	2·00 ± 2·34
Plant 6 .	103	79	24	77·25	25·75	1·75 ± 2·96
Total .	453	342	111	339·75	113·25	2·25 ± 6·22

The ratio in this case is 342 green to 111 yellow plants and the deviation is less than one third of the probable error. Hence " yellow " is a simple recessive to normal green. It has also been shown above (Table II) that factor pair (**Gg**) for yellow plant colour is not linked with the factor pair (**Aa**) which controls the production of anthocyanin in plants.

Inheritance of Bushiness

The bushy plants are characterised by having slender stems as well as a greater number of thin branches. They have a spreading habit of growth. There is no difference between the normal and the bushy when the plants are young but in the normal plants the flowering stalk becomes longer and the plants when mature can be easily separated.

TABLE IV

Segregation of bushy plants in the progeny of F_1 normal plants

Culture	Total No. of plants	Observed		Calculated		Deviation from 3 : 1
		Normal	Bushy	Normal	Bushy	
29·103	92	68	24	69	23	1·00 ± 2·80

The ratio in this case is sixty-eight normal to twenty-four bushy plants and the deviation less than one half of the probable error. This is a simple mono-factorial ratio ; therefore the character bushy must be conditioned by a single gene recessive to a gene responsible for the normal habit of growth.

GENERAL DISCUSSION

Unfortunately individual isolations of F_1 plants were not practised in both the cultures, therefore, no reliable data could be obtained on the colour of marginal achenes and hairiness *vs.* baldness in plants. At least plants with white marginal achenes should have been separated from those having coloured marginal achenes. In view of the fact that parent plants with white marginal achenes when crossed together gave an F_1 with coloured marginal achenes and the segregations in three of the F_2 families fitted so closely to 9 coloured : 7 white ratio, it is suggested that two complementary genes might be involved in the production of coloured marginal achenes. The occurrence of white marginal achenes in some of the F_1 plants shows that one of the parent plants was segregating for the complementary factor.

The objective in this work was to secure a strain of *C. dioscoridis* homozygous for all the recessive characters. There are four pairs of chromosomes in this species and it is desirable that each chromosome be differentiated by at least one gene effecting an easily distinguishable morphological character, in order that a further analysis of the species be made possible.

The studies of the comparative genetics of Drosophila and of rodents have proved that related species have germinal system which are either identical or very similar in organization. Collins [1924] crossed two glabrous plants of different races of *C. capillaris* from different localities and obtained all glabrous F_1 plants, proving that the same gene was responsible for the appearance of glabrous in each race. By crossing a non-hairy plant of *Crepis dioscoridis* with a non-hairy plant of *C. capillaris*, it can easily be demonstrated whether or not the recessive non-hairy is a case of parallel variation. The other recessive characters, anthocyanin absent and herbage yellow, which occur in this species, are also reported by Collins, to occur in *C. capillaris*. The occurrence of coloured marginal achenes is reported by Navashin [1929] in *C. aspera*. Thus there is evidence suggesting genetic parallelism in *C. capillaris*, *C. aspera* and *C. dioscoridis*. A parallel study of genetics and cytology of the three species and their hybrids might throw considerable light on their relationships.

The writer is indebted to Prof. E. B. Babcock at whose suggestion the investigation was started, and to Prof. R. E. Clausen and Dr. C. F. Pool for valuable help and suggestions during the progress of the investigation. Mr. C. W. Haney, Laboratory Assistant in the Division of Genetics kindly made the crosses and grew the F_1 plants.

SUMMARY AND CONCLUSIONS

1. In the species *Crepis dioscoridis* three pairs of factors *viz.*, **Aa, Gg** and **Bb** are found to be responsible for the inheritance of the characters, anthocyanin present or absent, yellow or green plant, and bushy or normal habit of growth respectively, and probably two pairs of complementary genes are involved in the production of coloured marginal achenes.

2. Plants of *Crepis dioscoridis* are largely cross-fertilized.

3. *C. dioscoridis* and *C. capillaris* exhibit similar variations in morphological characters and it is possible that they may be closely related to each other.

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A NOTE ON A NEW TYPE OF PROGRESSIVE DAMAGE TO THE STRUCTURE OF COTTON HAIR CAUSED BY MICRO-ORGANISMS

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(Received for publication on 16th March 1936)

(With Plates L and LI and one text-figure)

The ultimate transformation of cellulose matter into carbon dioxide and water through the agency of micro-organisms is an important balancing process of nature. The manner in which this process is sometimes associated with structural changes in cotton hairs appears to be hitherto un-recorded. Denham [1922] states, " Actual damage may take place either from the organism attacking the wall and reducing its effective thickness *at one or more points** or from bursting of the wall by the pressure produced by internal growth ". It follows that Denham's description and illustrations refer to reduction in wall-thickness at one or more points only. At these points, apparently, the micro-organisms gradually drill holes in the fibre and damage its structure. Complete disappearance of secondary cellulose in a certain length of the fibre leaving an almost empty cuticular tube is neither described nor illustrated by any previous worker in the available literature.

Burns [1927] recognizes two types of organismal deterioration, *viz.*, (1) Deterioration due to actual decomposition ; and (2) deterioration due to the presence and growth within the cotton hair of micro-organisms, which may develop to such an extent as to affect the physical properties of the hair by simple growth pressure. Although the deterioration described below may appear to fall in type 1 as above, yet, again specific instances of this nature are not given either in his description or the photomicrographs. Thaysen and Bunker [1927] and Prindle [1934], who have made a detailed study of the micro-biology of cotton hair and in whose publications full references to earlier work will be found, also do not make any mention of the type of damage described here.

In the course of examination by Bright's Congo red technique of Broach Palej cotton for damage due to storage, a new type of deterioration in which the cell-wall is reduced to cuticular thickness over varying lengths in different fibres, was noticed. The normal thickness of the cell-wall gradually tapers towards the hollowed portion as shown in the camera lucida drawing of one scuh fibre below. A remarkable feature, therefore, of this type of damage is that the nature of maturity chracteristic, *i.e.*, the wall-thickness, is radically changed in the affected portions from that of mature or half-mature into immature type. In order to verify this observation, actual measurements of wall-thickness and ribbon-width of ten fibres in the normal and corroded parts of each were made. These are given in Table I. It will be seen from the mean values that whereas in the case of normal portions the wall-thickness of these swollen hairs is nearly half the ribbon-width, it is less than 1/12th of the ribbon-width in affected portions.

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* The *italics* are mine.

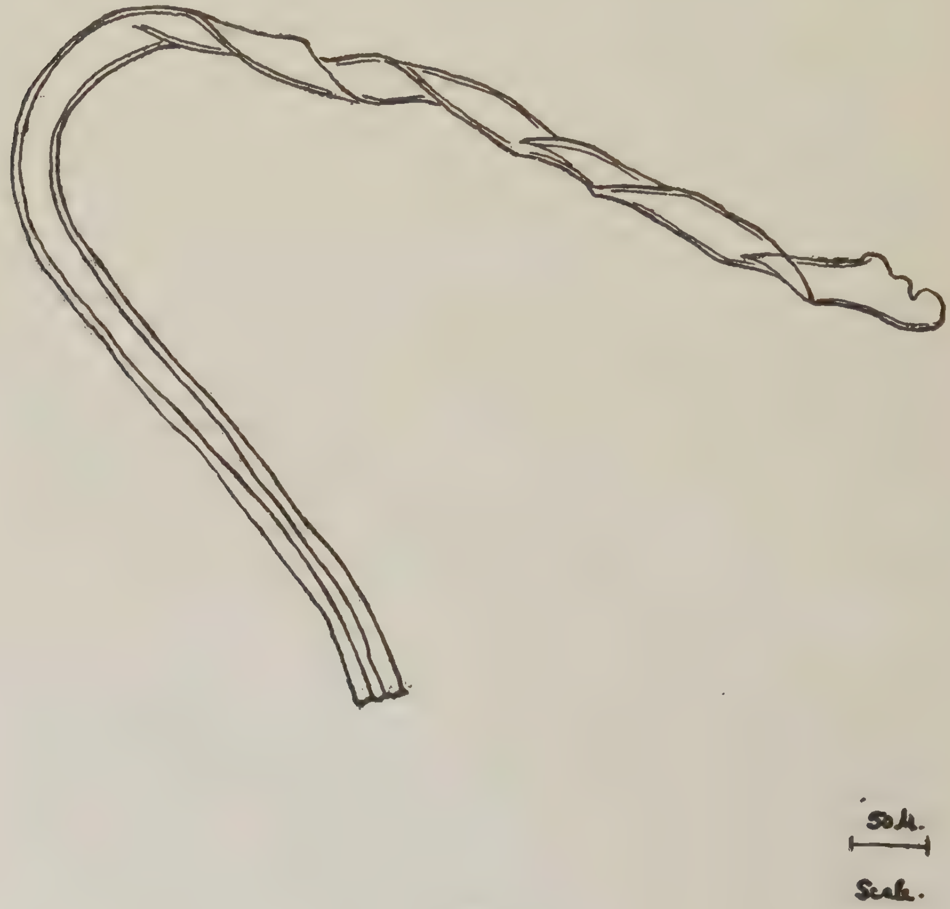


Fig. 1.

TABLE I

Fibre-number	Normal portion		Corroded portion	
	Ribbon-width	Wall-thickness	Ribbon-width	Wall-thickness
	Microns	Microns	Microns	Microns
1	32·71	13·75	35·31	2·61
2	24·88	12·32	26·68	1·90
3	36·02	17·06	32·23	2·37
4	23·70	11·61	25·36	2·13
5	18·49	7·58	18·49	2·37
6	26·31	9·24	33·42	3·56
7	23·70	9·71	28·44	3·79
8	22·51	10·67	23·46	1·42
9	30·81	9·48	31·05	1·90
10	29·62	10·67	34·60	2·13
Total .	268·75	112·09	289·04	24·18
Mean .	26·87	11·21	28·90	2·42

This damage is wholly internal in contrast to the two types already described which sooner or later lead to external injruy.

The original sample of this cotton before storage showed no instance of internal corrosion ; while the sample stored for six months showed about ten per cent of such hairs ; and after twelve months the corroded hairs were as numerous as twenty-five per cent. (In each case 300 fibres were examined from a representative sample). The increase in the number of affected hairs and also the affected length indicate that the change has been brought about during the period of storage.

The internal corrosion usually occurred towards the basal end of the fibre. In a few cases, however, it was noticed near the apical and also, when the latter happened to be broken. Again, instances of this hollowing process from the injured spot on either side of the injury were also observed. The maximum length affected in this manner extended up to one cm. from the broken end.

The reason why such damage occurred most commonly at the basal end is that the fibre is torn from the seed-surface, resulting in an opening in the lumen. This opening admits micro-organisms to the interior of the otherwise closed tube. Once the organisms enter the lumen, they not only obtain dried protoplasm as food material to thrive on, but also come in direct contact with the internally unprotected cellulose. The moment they establish themselves, they commence functioning as cellulose decomposers, if they happen to be of that type. They cannot do it as easily from the outside on account of the presence of a protective waxy layer of cuticle on the fibres.

Examination of numerous fibres (5,000) from end to end showed that the internal corrosion of cellulose is invariably associated with the presence of micro-organisms. Some seventeen species of fungi and three types of bacteria were isolated from this cotton. These will be described in a later publication. It is not easy at this stage to assign the internal corrosion to any one of these micro-organisms. Possibly both bacteria and fungi are involved in this process. The hollowed out parts were found to be localised near the broken ends or injury spots both of which are well-known seats of organismal infection. Furthermore, these parts were found to stain deep red by the Congo Red technique which according to Bright [1926] indicate moderate fungal damage. This, however, may be due to the action of bacteria, as Bright's technique is unable to distinguish between fungal and bacterial damage. The absence of secondary cellulose near the basal end of mature hairs is inexplicable on any other ground except as a result of the action of micro-organisms. Any interruption in the flow of protoplasm which is responsible for the deposit of cellulose in layers must essentially affect the rest of the fibre, as the source of nutrition is below its base. The fibres in question were, however, found to be quite well developed above the affected lengths. An alternative explanation on the basis of shrinkage of cellulose but not of cuticule may even be considered. This is also contrary to the observation of Osborne [1935] that cuticle shrinks in length on being treated with caustic soda and in circumference on being washed in water after the process of mercerisation. The cell-wall was usually found to taper towards the hollow portions in which lay disintegrated layers of cellulose wall. In the event of mere shrinkage there is no reason for the cell-wall to taper or lose its compactness. It is, therefore, concluded that internal corrosion of cotton fibre is wholly attributable to the decomposing action of micro-organisms.

In support of the above view nine photo-micrographs are reproduced below. Plate L, fig. 1 shows the basal end of an undamaged hair. Plate L, fig. 2 shows the beginning of the internal corrosion which extends up to the point marked by the letter A. In Plate L, fig. 3 the damage has progressed to about 0·7 mm. Fig. 4 shows a still greater length affected in this manner, the letter A again marking the extent to which corrosion has progressed. Plate L, fig. 4 shows corrosion on either side of injury at B occurring about the middle of a fibre. Plate L, fig. 5 brings out clearly that the cell-wall tapers from the normal to the corroded region. Plate LI, fig. 1 shows the details of the affected part. The presence of some spirillar organisms as indicated by the arrow may be noted. Plate LI, fig. 2 illustrates organisms in the lumen of an otherwise normal portion of cotton fibre. This fibre looks highly magnified as it was flattened out by pressure in the course of examination. Plate LI, fig. 3 relates to two fibres, a thin one and a broad one, the latter showing, in its interior, dislocated layers of cell-wall, which precede corrosion at the transition point between the normal and the affected regions. Plate LI, fig. 4A shows partly peeled off layers from the cell-wall, while 4B shows some of the organisms, at a high magnification, in the corroded region.

Fig. 1 (×100)

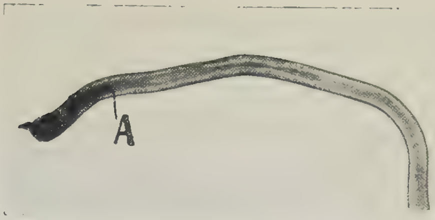


Fig. 2 (×100)

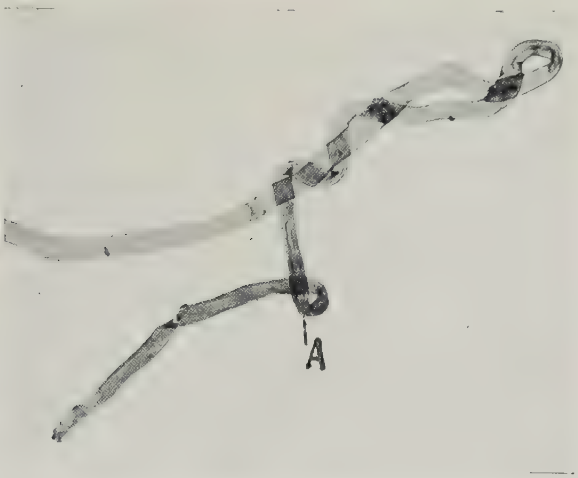


Fig. 3 (×100)

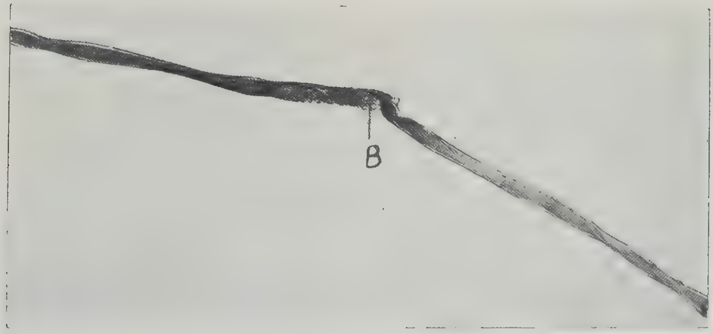
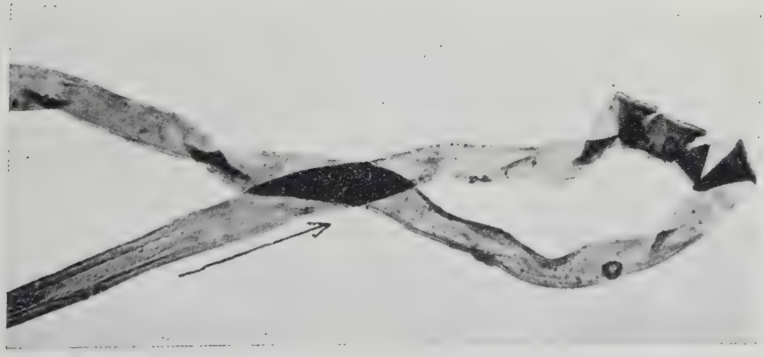


Fig. 4 (×100)



F 5 (×200)

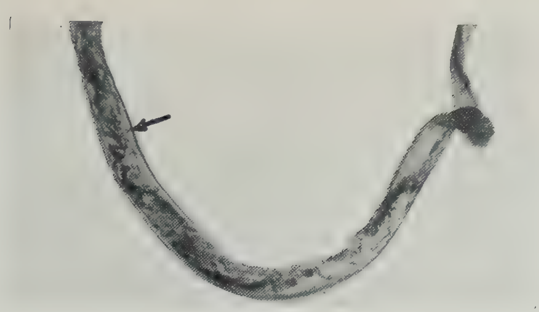


FIG. 1 (×180)

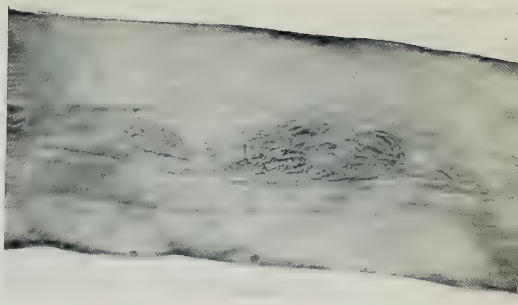


FIG. 2 (×325)

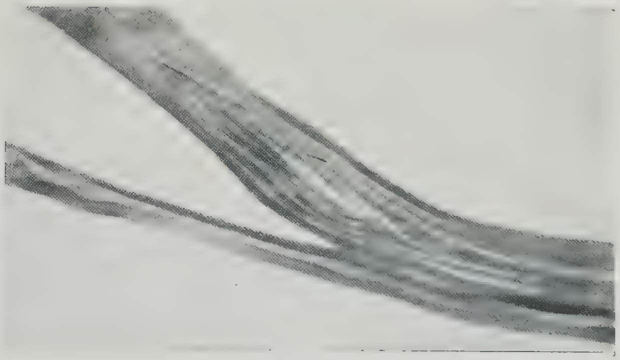


FIG. 3 (×325)

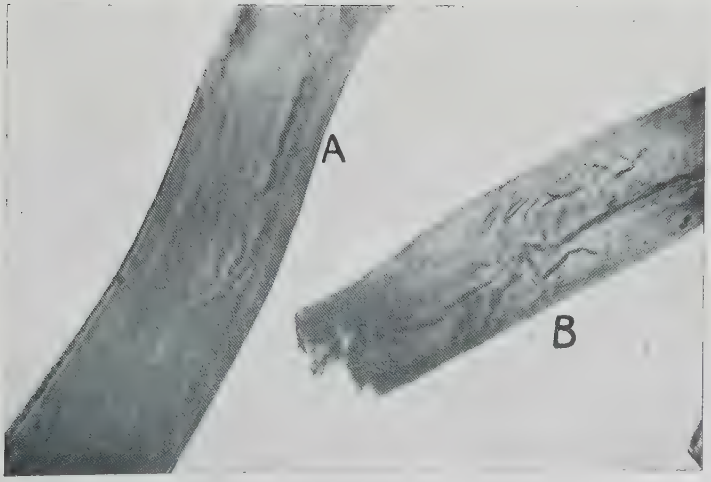


FIG. 4 (×700)

SUMMARY

While investigating the causes of deterioration of Broach cotton, a type of damage to the structure of cotton fibre was discovered which does not appear to have been described by the earlier workers. The chief characteristics of this damage are that (1) it commences from the usual seat of organismal infection, *i.e.*, the basal end or a tear in any other part of the fibre ; and (2) it slowly progresses internally disintegrating and corroding the cell-wall, leaving intact merely cuticular sheath. The cell-wall gradually tappers from the unaffected portion into the affected one. Nine photo-micrographs are reproduced in support of the observation.

In the end the writer takes this opportunity of expressing his gratitude to Dr. Nazir Ahmad, Director of the Laboratory for his helfpul guidance.

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SELECTED ARTICLE

METHODS OF ANALYSIS AND APPRECIATION OF WHEAT, FLOURS AND BREAD, PARTICULARLY IN RESPECT OF INVESTIGATIONS ON THE BAKING VALUE

BY

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(Reprinted from the *International Review of Agriculture, Monthly Bulletin of Agricultural Science and Practice, Rome*, Year XXVI, No. 9, September, 1935)

(With Plate LII)

FOREWORD

The numerous researches recently made in regard to the chemistry of cereals by well-known scientists and investigators throughout the whole world have led to considerable changes in analytical chemistry and have shown the necessity for bringing up to date and unifying the methods of analysis and research. Ing. Arnaldo Luraschi, President of the " Federazione Nazionale dei Panificatori ", made this necessity clear at the 1st International Bread-making Congress (Rome, 1932) and his proposals were again vigorously defended at the 3rd International Technical and Chemical Congress of Agricultural Industries (Paris, 1934) and at the 16th International Agricultural Congress (Budapest, 1934).

It is certain that great improvements would be effected in the cereal trade if it were possible to come to an international agreement on the processes and methods of analysis and estimation. This, however, is a very delicate and complex question which is more difficult to solve than would appear at first sight. In fact, it is not only a question of standardising present methods, but it is above all essential that analysts and investigators should put their own personal feelings on one side and work together with the same methodical and scientific spirit.

The object of this present article is merely to present and to specify the most recent and accurate methods of research in the hope that an international meeting of scientists, technicians and persons interested in the question will be held in the near future and will lead to an agreement, and also to a practical application as indicated above.

To return to the subject, according to the object of the research the method of analysis may be classified in three categories :

(1) analysis of wheat ;
(2) analysis of flours ;
(3) experimental tests and analysis of bread.

It is certain that, with regard to the baking value, the most satisfactory determination is obtained by the practical proof of baking. In fact, the quality of the bread obtained allows an indirect, but exact, estimate to be made of the baking " strength " of the flour.

It must, however, be added that analytical research is always necessary as not only does it demonstrate the various chemical, physical and physico-chemical properties of flours and the origin of certain phenomena, but also may provide elements whereby it will be possible rapidly to appreciate the qualities of the product examined.

In this first chapter research on wheat will be discussed and in the following chapters flours and bread.

CHAPTER I

Analysis of wheats

Wheats have very variable characteristics and properties, dependent on numerous factors which are connected not only with the variety, but also with the systems of cultivation, the nature of the soil, climate, etc.

There are a great number of varieties of wheat, several tens of thousands, and the number increases continually owing to the work of selection and hybridisation.

Following the production and trade classification wheats may be placed in three large categories :—

(1) Hard wheats (*Triticum durum* Desf.) with hard and heavy grain, compact and flinty on breaking.

To this category belong the hard wheats of strong bread-making quality of the types Manitoba, Bankuti, etc., and also the wheats with yellow, golden or straw coloured grain chiefly suitable for the manufacture of alimentary pastes. These latter are grown principally in hot countries.

(2) Semi-soft wheats (*Triticum turgidum* L.), average sized grains, yellowish in colour, on breaking starchy in the centre and flinty on the outside. In Italy these wheats are grown in the central regions.

(3) Soft wheats (*Triticum vulgare* Willd.) differentiated from the preceding wheats by the fact that the grains break readily and are white and starchy throughout.

As has already been seen, not all wheats are suitable for bread-making ; while many soft and semi-hard wheats have the characteristic qualities for the preparation of light and well-risen bread, a certain number of hard wheats, particularly suitable for the manufacture of alimentary pastes, have properties which render them unsuitable for bread-making.

A flour, to be really suitable for bread-making, should have a combined set of characters which are known in the trade as the " strength of the flour ". This is the combination of chemical, biochemical, physical and physico-chemical properties which, on the one hand, give a normal and adequate fermentation and, on the other, impart to the dough a sufficient resistance to the pressure of gases disengaged as to confine them as much as possible. Hence the mass dilates and its volume expands uniformly and a bread is obtained which is large-volumed, light, appetising and perfect in all respects.

Wheats and flours are known as "strong" when they possess the properties indicated to an adequate degree while the "weak" wheats and flours are characterised by an insufficiency of these properties.

Passing on the analysis of wheat, a few brief words will now be said on the principal determinations, at the same time particularly emphasising the latest improved methods.

The present methods of appreciating wheat may be classified under four headings :—

(1) Methods for determining physical and organoleptic characteristics.
(2) Methods of investigating the chemical properties.
(3) Methods based on biochemical determinations.
(4) Methods relating to the yield in flour.

It is, however, advisable to first say a few words on the taking of samples, grinding and preparation of the product.

Taking of samples.—The essential condition for obtaining accurate results in the analysis of cereals is to take samples which represent an average of the product examined. For this purpose suitable apparatus are introduced into the mass of grain and several samples are taken from various depths. These are then mixed together into the shape of a square. This square is divided into four parts by the two diagonals. Two of these parts, the opposite angles of which touch, are then taken. If the sample is too large the above process is repeated until a sample is obtained suitable for research purposes.

Grinding and preparation of the product.—Before being subjected to chemical analysis, the wheat should be ground so that it constitutes a fine and homogeneous mass, uniform and constant in composition. For this purpose a small laboratory mill is required and the grains should be ground in such a manner that the chemical composition of the product is not altered. This work must, therefore, be carried out slowly, avoiding any heating of the flour which would affect the composition. The flour thus obtained is conserved in glass pots hermitically sealed with parafin until such time as they are utilised for further investigation.

A. The study of the organoleptic and physical properties of wheat, which are of great importance from the scientific and practical point of view, relate to the following characters :—

(1) Purity of the wheat ;
(2) Weight of 1,000 grains:
(3) Volume of 1,000 grains ;

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(4) Weight per hectolitre ;
(5) Specific weight ;
(6) Flintiness or hardness.

Determination of purity.—The purity of the wheat is a valuable factor for the classification of the wheat from the standpoint of commercial value. It would be desirable to be able to fix a maximum tolerance of the total quantity of foreign bodies (2 per cent for examples), as these foreign bodies are the cause of considerable loss during the processes of cleaning, especially if they are in the form of particles of iron, small stones, etc.

The purity of the wheat is determined in a very simple manner ; after having taken a sample of 100 grammes of wheat, all the foreign substances are separated from the mass by means of a magnifying glass and a pair of small pincers. The sample is then weighed again and brought up to 100 grammes.

Weight of 1,000 grains.—The weight, which is in direct relation to the size of the grains, is generally determined by means of a special grain-counting apparatus.

Volume of 1,000 grains.—In the majority of cases there is a direct relation between the volume of the grains and the yield in flour. The volume of 1,000 grains is determined by means of a small pycnometre fitted with a graduated tube. The apparatus is rilled with xylol, 1,000 grains are introduced and the volume may be immediately read off from the graduated tube. The data obtained are very satisfactory from the practical point of view. If more accurate results are required, a volumenometre must be used which will be mentioned again later when the specific weight is discussed.

Weight per hectolitre.—Special importance is attached to this point in the purchase and sale of wheat although it does not precisely indicate the true milling yield. It should be noted here that the true commercial value of wheat is to be estimated not only according to the yield in flour, but also according to the use to which it will be put. It has in fact been ascertained that the milling value of wheats is shown approximately by the weight per hectolitre, while there is no relation between this weight and the suitability of the wheat for bread making. Several factors influence the value of the weight per hectolitre, among which are the following :—

(1) The space occupied by the wheat which is dependent on the close packing between one grain and another.
(2) The specific weight.

These factors, in their turn, are dependent on : the chemical composition of the grain, and especially on its content in water and bran ; the volume ; the shape ; the presence of foreign bodies and imperfect and unripe grains ; the smoothness of the surface of the grain on which depends the slipping down or heaping up of the mass. It is understood also that the variation in the weight per hectolitre is also influenced by the soil and climatic conditions of the place of origin of the crop, by the degree of ripeness, or of dryness, by the purity, etc. To these factors, which are dependent on the quality of the product, must be added other factors related to the methods employed for warehousing the wheat. The weight of the hectolitre varies considerably according to the height and capacity of the warehouse and the quantity stored, and it is thus that, according to different methods of storing, (sacks transported and emptied by hand, "transporter" elevators, suction elevators, etc.) the heaping of the grain will vary and with it the weight per hectolitre.

In the trade it is more important to obtain values which are comparable and correspond to each other than very accurate results; also it is essential strictly to follow the prescribed rules and to employ the methods and apparatus officially adopted. The Schopper apparatus with a capacity of one litre is the most utilised; special tables give the weight of the hectolitre calculated from the figures shown on the scale. It is advisable for this determination to place the wheat in the apparatus by means of a filler of funnel shape with a wide base so as to obtain the same packing each time*.

The weight of wheat to the hectolitre varies from 68 to 84 kg. and is as much as from 87 to 88 kg. for hard wheat.

Specific weight.—The value of this weight varies from 1·28 to 1·50 and is in strict relation to the quantity of the cereal examined.

In the opinion of certain authors, the specific weight of a wheat should always be determined in order to appreciate the commercial value as this would be much more accurate than the weight per hectolitre the determination of which is influenced by various factors.

The specific weight varies according to the differences in the nature of the product examined and, with regard to cereals, two factors must be taken into consideration :—

(1) The air content;

(2) The chemical composition of the cereal.

Air is present either in the external integuments or in the albumen; the specific weight diminishes as the air content increases. Humidity greatly influences the specific weight, also the protein substances, there being a diminution in the specific weight in proportion to an increase in these substances.

For determining the specific weight of cereals, the apparatus used at present are ordinary pycnometres or special pycnometres (Bruckner-Kuhn apparatus) and the method of heavy liquids of Prof. Neumann is followed (Zeitschrift fur das gesamte Muuhlewesen, 1927,4). These processes are not, however, free from error as the xylol, as well as the other liquids, does not adhere perfectly to the surface of the grain and is also more or less absorbed by the grain.

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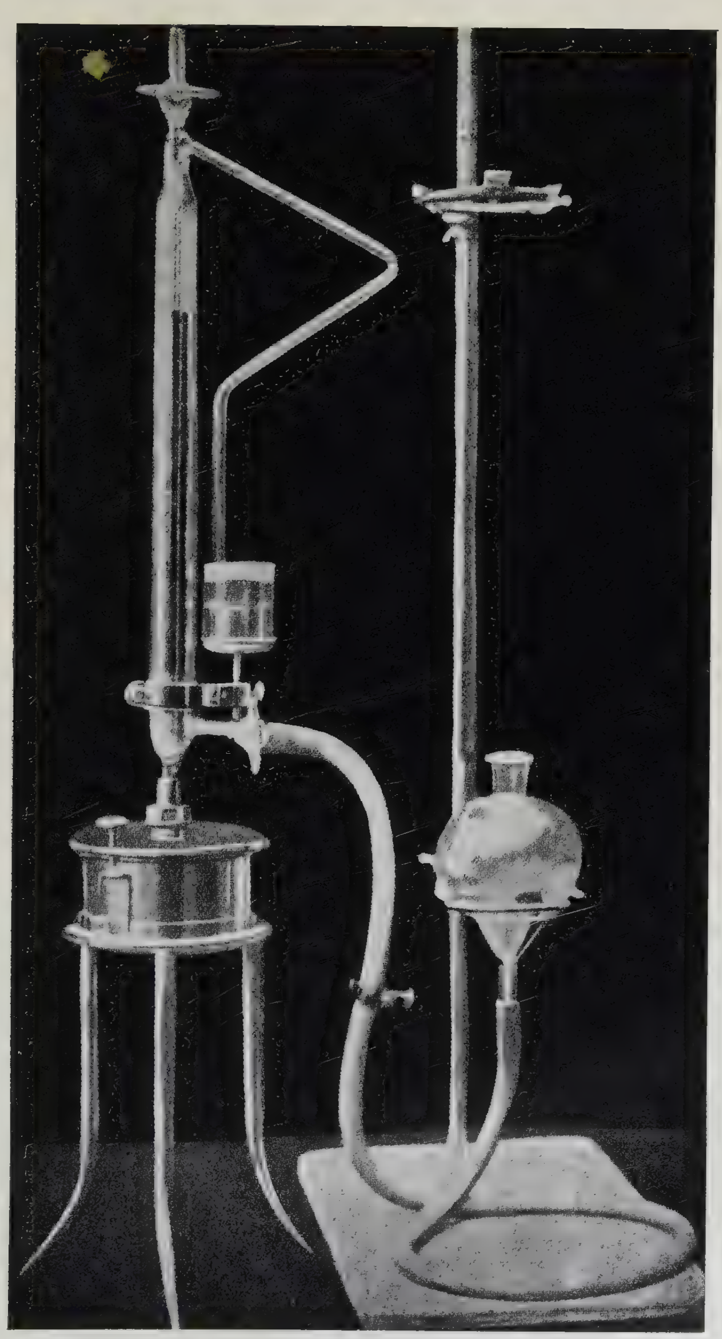
* E. Schmidt has invented a new appratus whereby by means of a system of small gratings the packing of the wheat in the receptacle is simplified and is more uniform and constant. The results are higher, but more in agreement and more accurate (Das Mihlenlaboratorium, 1932, 4).



Volumenometre with direct reading.

The most accurate method, and the one that should be adopted for cereals, is certainly that which is based on the use of volumenometres ordinarily employed in physics for determining the specific weight of substances with a high absorbent capacity.

In our laboratory we have used a volumenometre invented by our collaborator, Dr. De Rege, which has the advantage of giving a direct reading. The characteristic of this apparatus is that the glass tube containing mercury is graduated so as to correspond to a given barometric depression. The calculation is very rapid. The apparatus is calibrated, the recipient is filled with a certain quantity of grain and the result is read and verified by means of the manometre. The total specific weight is obtained by dividing the number of cubic centimetres occupied by the cereal by the weight of the cereal.

Specific weight of various wheats

Wheats	Pycnometre		Bruckner Kuhn apparatus (Xylol)	Method of Prof. Neumann	Volumenometre	Weight per hectolitre (in hg.)
	Water	Xylol				
Amber	1,250	1,378	1,377	1,384	1,410	82·15
Senatore Cappelli	1,224	1,335	1,334	1,360	1,385	78·60
Manitoba	1,256	1,355	1,356	1,382	1,415	83·70
Mentana	1,248	1,402	1,400	1,385	1,419	80·35
Balilla	1,249	1,355	1,352	1,374	1,410	80·35
Virgilio	1,234	1,355	1,355	1,375	1,416	78·60
Catria	1,258	1,332	1,330	1,371	1,399	78·80
Zara	1,230	1,355	1,350	1,375	1,400	81·23
Fausto Sestini	1,257	1,333	1,330	1,375	1,420	76·80
Damiano Chiesa	1,268	1,334	1,333	1,372	1,400	81·25

The data given by the volumenometre are higher, but more accurate. It must also be remarked that there is no strict relation between the value of the specific weight and the weight per hectolitre.

Determination of flintiness.—According to a great number of authors, the degree of flintiness or hardness is in direct relation to the quantity of nitrogenous substances and gluten contained in the wheat. The wheats richest in flinty grains are the most sought after for making alimentary pastes, while those richest in starchy grains are most suitable for manufacturing starch. It must be remarked that there are hard wheats which are not suitable for making pastes, while being eminently suitable for bread-making.

For the purpose of determining the hardness of wheats, special apparatus for taking sections are used and the percentage of hard, semi-hard and starchy grains is calculated.

B. Chemical analysis of wheat generally includes the following determinations :—

(*a*) Determination of humidity,
(*b*) Determination of ash,
(*c*) Determination of proteic substances,
(*d*) Determination of fats,
(*e*) Determination of crude cellulose.

Determination of humidity.—Humidity is an element of great importance in estimating the value of wheat from the commercial standpoint and from that of its keeping qualities. The humidity content of wheat is chiefly dependent on the climatic conditions in which it has been grown. Humidity appears under various forms, especially :

Absorption humidity ;
Vegetation humidity ;
Humidity of chemical constitution.

The first is derived from the soil, the second is due to rains, mists, dew, etc.

Vegetation humidity is present in all the phenomena of development, growth and maturation of the plant. The cereal loses a part in drying and retains a quantity varying from ten to sixteen per cent.

As to the third type of humidity, constituted by the water of crystallisation and that derived from the disintegration of molecules, no investigation has been made.

Several methods are followed for determining the humidity. In our laboratory the process is as follows :—

Five grms. of wheat flour are taken and placed in a calibrated weighing bottle, it is then dried in a stove at 105°, up to constant weight (six hours)*.

Determination of ash.—The ash content in wheats varies from 1·5 to 2·6 per cent and the wheats poorest in ash are generally the least rich in cellulose.

For the determination of ash the process is as follows :—Five g. of wheat flour is carefully weighed in a platinum or porcelain evaporating dish and then placed in a muffle furnace which is progressively heated up to a maximum temperature of 650°. For rapid results it is advisable to moisten the mass after carbonisation with small quantitites of oxidising substances such as neutral oxygenated water, a watery solution of ammonium carbonate or nitrate, etc.

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* The determination of humidity in a stove at 105° does not give very accurate results and as a rule there are errors of about 0·3 per cent. To eliminate completely the last traces of humidity a Liebig U-tube must be used, brought up to 105° and through which a current of dry air or inert gas is passed. The rapid methods recommended lately such as those based on the use of high temperatures, or on the determination of water by distillation in the presence of special liquids, or on the determination of humidity by electric constants, although of great interest on account of their rapidity, do not give an absolutely accurate determination.

Determination of nitrogenous substances.—The content of wheat in nitrogenous substances is in direct relation to the gluten in the flour and varies greatly from one wheat to another. With certain hard wheats it is as much as ninteen per cent.

The quantity of nitrogen is ascertained by the Kjeldahl-Ulsch method, using 3 gm. of flour, 25 ccm^3 of phospho-sulphuric acid (125 gm. phosphoric anhydride dissolved in 1,000 ccm^3 of sulphuric acid at 66°B), a small quantity of copper oxide (0·05 gm.) and four to five drops of a ten per cent solution of platinum chloride. The quantity of protein substances is obtained by multiplying the nitrogen content ascertained by the factor 6·25. The American official method prescribes the factor 5·7, while the figure 6·25 is generally adopted in European laboratories.

Determination of fats.—Ten gm. of flour are placed in an extraction filter and dried for two hours in a hot water oven. Extraction is then carried out for ten hours in a De Soxhlet apparatus with anhydrous ether. The filter is removed and the balloon flask containing the ether extract is heated to eliminate the ether. The final traces of ether are removed by heating the open balloon flask in a bain-marie. The residue is then dried for one hour in a hot-water oven and then weighed. The fat content varies greatly in different wheats, and may be from 1·5 to 1·9 per cent.

Determination of crude cellulose.—For this operation the Weende method is the most advisable. The procedure is as follows :—

In a porcelain evaporating dish is placed 3 gm. of flour with 200 cm^3. of sulphuric acid (1·25 per cent) and boiled for 1½ hours, adding water as evaporation takes place. It is then filtered and the contents of the filter is collected in the evaporating dish by a jet of water ; 200 cm^3 of water is added and the whole is boiled for half an hour. It is then filtered again, the water treatment is repeated ; it is boiled again and the residue is placed in the filter.

The residue in the filter is boiled again in the same evaporating dish for half an hour with 200 cm^3 of a solution of caustic potash (1·25 per cent) and filtered. The water treatment is repeated twice, boiling each time for half an hour.

All residue not dissolved is passed through a calibrated filter, previously dried at 105°, it is first washed in boiling water then cold water, and finally in a mixture of alcohol and ether, it is then dried at 105° and weighed. The filter and its contents are placed in a crucible, the whole is incinerated and weighed. The quantity of crude cellulose, referred to 100 parts of substance, is obtained by deducting from the weight first obtained the weight of the ash. The proportion of crude cellulose varies from 1·5 to 3 per cent and even 4 per cent for certain wheats.

C. Bio-chemical tests, which are made on the whole wheat, include all the methods whereby the baking value of the variety may be determined with more or less accuracy.

The need for a method suitable for the requirements of farmers and geneticists (who, for the creation of new varieties, must carry out tests on small quantities of wheat) has resulted in new studies being made by numerous investigators.

The method most widely used by technicians is certainly that of Dr. Pelsehnke of Halle University. (Archiv fur Pflanzenbau, 1930, 5). This scientist has introduced a bio-chemical process called "Schrogarmethods" for determining the quality of wheat from the point of view of bread-making. The process is as follows :—

Ten g. of whole flour is kneaded with 0·5 gm. of yeast and 6 cm^3 of water so as to obtain a dough of average consistency. Contrary to what usually occurs with ordinary flour, whole flour, according to the writer, will always have the same degree of water absorption, so that the tests will give constant results.

The dough is divided into two equal parts and well kneaded by hand on a glass sheet so as to form two balls of dough, taking care to make them perfectly homogeneous. These two balls are placed in two glasses 6 cm. in diametre and 7 cm. in height, and filled with 75 cm^3. of water. These are placed in a thermostat at 32°. The time when the balls are placed in the glasses is noted and also the moment when, after the splitting of the dough into fragments these fall to the bottom of the glass. The time, in minutes, which elapses between the beginning of fermentation and the break up of the dough is taken as an index figure of the quality of gluten. Weak flours break up rapidly, strong flours slowly.

These index figures vary greatly according to the variety of wheat. The finest wheats have indices exceeding eighty ; the weakest have indices below thirty.

This method, which according to numerous experimenters gives results very fairly in agreement with baking tests, is particularly valuable in the field of genetics with regard to the choice and estimation of new varieties.

Another rather similar method is the Guttingen (Das Muhlenlaboratorium, 1934, 7 and 10). The increase in volume of the ball of dough is determined during fermentation and consequently the capacity of the dough to retain gases. This property is more pronounced in the flours of superior quality.

D. Investigations on the milling yield.—An approximate appreciation of wheat from the point of view of milling is arrived at in the trade, on the basis of weight per hectolitre.

It is generally considered that wheats having a weight per hectolitre less than 80 give a yield in flour of less by 1 to their weight per hectolitre, while the wheats with a weight per hectolitre higher than 80 give a yield in flour of less by 2.

This naturally only serves as an indication and the desirable precision cannot be obtained in this way as other factors, such as humidity, size and shape of the grains, percentage of foreign bodies, etc., also have an influence.

Thanks to modern methods of milling, the miller obtains several qualities of flour from the wheat which are sold under different names according to the choice of the producer and the purposes for which they are intended.

With a view to judging the milling quality of a wheat, millers take as a basis the various qualities of flour obtained in identical conditions. It must be remarked that the yield in flour also depends, in addition to the quality of the wheat, on the system of milling and the improved character of the milling apparatus.

Investigators in the laboratories extract a single quality of flour known as whole or complete flour, obtained from wheat with the bran removed, extraction being effected in apparatus of reduced size and according to different methods.

It is certain that the results thus obtained cannot agree. In fact, scientific and practical methods must be followed which give results corresponding to and in agreement with those obtained at the mill.

For this reason we have adopted a small experimental roller mill called the "Vottero experiment mill" and with which we obtain very satisfactory result.

MYCOLOGICAL NOTE

ON THE SYSTEMATIC POSITION OF THE SMUT CAUSING MALFORMATION OF *VITIS QUADRANGULARIS*

BY

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Imperial Institute of Agricultural Research, Pusa

(Received for publication on 21st February 1936)

In 1922 Iyengar and Narasimhan [1922] reported a smut from Madras on *Vitis quadrangularis* Wall., which caused a distinct witches' broom of the host. The disease started at the nodes where the diseased parts branched repeatedly and irregularly, bearing dark-green swellings which, when cut open, showed dry, black, powdery mass of spores. The spores were coupled in pairs and possessed a dark-brown, smooth epispore ; the authors were unable to induce spore-germination. The smut was described as a new species of *Schizonella* and given the name *S. colemani.*

The genus *Schizonella* was created by Schroeter in 1877 to describe a smut whose sori are formed in the leaves and whose spores occur in pairs, being joined together in the middle by a kind of isthmus. The spores form *agglutinated* masses and are *serially* produced in the fertile hyphae. Clinton [1904] states that both Schroeter and Brefeld had succeeded in germinating the spores of the species. The species of *Schizonella* so far described seem to be confined to temperate climate and to the family Cyperaceae.

Beck erected another genus, *Mycosyrinx*, to describe the tropical smuts with paired spores. The sori in the case of this genus occur in the interior of the pedicels and the peduncle causing a witches' broom and contain a *powdery* mass of spores. The spores are produced *singly* at the ends of the fertile hyphae and their germination has not so far been observed. The genus seems to be confined to the family Vitaceae and to the tropics. Only two species have so far been described : *Mycosyrinx cissi* (DC) Beck and *M. arabica* (Henn.) Penzig. The latter of these is reported on *Cissus quadrangularis* which is a synonym for *Vitis quadrangularis* Wall.

The writer has seen Iyengar and Narasimhan's type specimen (part of which, kindly given to him by Professor M. O. P. Iyengar, is in the Pusa Herbarium) and it is manifest that the part of *V. quadrangularis* that is affected is the peduncle. The spores are in a *powdery* mass and their measurements are in agreement with those given by Penzig. There seems to be no doubt that the smut belongs to the genus *Mycosyrinx* and not to *Schizonella.*

The species of *Mycosyrinx* (*M. arabica*) mentioned above as parasitic on *Vitis quadrangularis* is known to occur in India ; it was collected by Dr. W. McRae at Mettupalayam, Madras Presidency, in 1911 and was identified by Sydow [1914]. A herbarium specimen of the smut which exists at Pusa, has been compared with Iyengar and Narasimhan's specimen and the result of the comparison leaves no doubt that the smut named by them as *Schizonella colemani* is identical with *Mycosyrinx arabica* (Henn.) Penzig and that the former name should therefore be abandoned.

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NOTES

TABLES OF STANDARD ERRORS OF MENDELIAN RATIOS

Tables of Standard errors of Mendelian ratios have been prepared for the 1 : 1, 3 : 1, 9 : 7, 15 : 1, 13 : 3, 27 : 37 and 63 : 1 ratios, in the Botanical Section of the Imperial Institute of Agricultural Research, Pusa, by Dr. S. S. Purewal and Mr. P. Krishna Rao, and have been published as Miscellaneous Bulletin No. 11 of the Imperial Council of Agricultural Research.

These tables give directly the standard errors for values of n from 5 to 1,000, and can also be used for determining any value of n beyond 1,000 and up to 100,000 by moving the decimal point and by simple linear interpolation where necessary. The method of using the tables is simple and is explained in the introductory note which precedes them. They should prove of considerable use to plant breeders and geneticists.

B. P. P.

PROCLAMATION OF THE UNION OF SOUTH AFRICA GOVERNMENT

Proclamation 290 of 1930

* *No. 290, 1930*

Whereas Mosaic Disease of Sugar Cane, being a plant disease in terms of the Agricultural Pests Act, 1911 (Act No. 11 of 1911) has been found to exist in the areas enumerated in the Annexure hereto ;

And whereas it is deemed to be necessary to impose restrictions for the prevention of the spread of the disease ;

Now therefore under and by virtue of the powers in me vested by paragraph (*c*) of section fourteen of Act No. 11 of 1911, I do hereby declare, proclaim and make known that the prohibition and restrictions set out in the Annexure hereto shall exist in respect of the removal of the plants therein named, to the extent therein provided.

Any person guilty of a contravention of any such prohibition or restriction, or failure to comply with any provision of this Proclamation with which it is his duty to comply, will, under section twenty-seven of the said Act, be liable to the penalties in that section mentioned.

Proclamation No. 12 of 1927 is hereby repealed as and from the date of publication of this proclamation.

God Save the King.

Given under my Hand and the Great Seal of the Union of South Africa at Pretoria this Twenty sixth day of November one thousand Nine hundred and Thirty.

ATHLONE,
Governor-General.

By Command of His Excellency the Governor-General-in-Council.

J. KEMP.

ANNEXURE

1. For the purpose of this Proclamation the following are declared to be restricted areas :—

The Province of Natal and Zululand, excluding the following Magisterial Districts of Northern Zululand:

Ubombo and Ingwavuma.

2. From and after the date of publication, no person shall remove or cause to be removed, except by the special permission of an officer appointed by the Minister of Agriculture, any sugar cane plant or any part of sugar cane plants of any variety other than the varieties Uba, Ch 64/21, P. O. J. 2878, and P. O. J. 2725 from any place within the restricted area to any other place either within that area or outside thereof.

The expression "variety Uba" means any sugar cane plant which has the characters of the leaf and stalk of the cane recognized in Natal as Uba and has not been raised from seed cane derived from any plot or field which the Government Mycologist has certified to be wholly or in part infected with Mosaic disease.

The expression "variety Ch 64/21" means any sugar cane plant which has the characters of the leaf and stalk of the cane recognized by the Government Mycologist as Ch 64/21 and which has been derived from the seed cane which was imported from Cuba under the name of Ch 64/21 by the issue of the Plant Importation Permit No. 89 of 15th March, 1926.

The expression "variety P. O. J. 2878" means any sugar cane plant which has the characters of the leaf and stalk of the cane recognized by the Government Mycologist as P. O. J. 2878 and which has been derived from seed canes which were imported from the Incomati Estates, Portuguese East Africa, under the name of P. O. J. 2878 by the issue of Plant Importation Permits Nos. D. 585 of 21st October 1927 and D 719 of 20th September 1928.

The expression "variety P. O. J. 2725" means any sugar cane plant which has the characters of leaf and stalk of the cane recognized by the Government

Mycologist as P. O. J. 2725 and which has been derived from the seed canes imported from the Department of Agriculture, U. S. A. and from Incomati Estates, Portuguese East Africa, under the name of P. O. J. 2725 by the issue of Plant Importation Permits Nos. D 504 of 9th March, 1927, and D 585 of 22nd October 1927, respectively.

PROCLAMATION 290 OF 1930

Amendments to regulations

* *No. 2192* (*5th December 1930*)

For the purpose of dealing with the outbreak of Mosaic Disease in sugar cane in the areas restricted by Proclamation No. 290 of 1930 the Governor General has been pleased, in terms of section *twenty-eight* of the Agricultural Pests Act, 1911 (Act No. 11 of 1911) to make the following regulations :—

Regulations

1. The regulations published under Government Notice No. 64 of the 14th January, 1927, are hereby repealed as and from the date of the publication of the regulations hereunder.

2. For the purpose of these regulations the expressions "variety Uba" "variety CH 64/21" "variety P. O. J. 2878" and "variety P. O. J. 2725" shall be as defined in the Annexure to Proclamation No. 290 of 1930.

3. From and after the date of publication of these regulations no person shall plant or cause to be planted in any area restricted by Proclamation No. 290 of 1930, which areas are set out in the schedule to these regulations, any sugarcane of a variety other than the varieties Uba, CH 64/21, P. O. J. 2878 and P. O. J. 2725 without a written permit from any officer appointed by the Minister of Agriculture.

4. From and after the date of publication of these regulations no person shall keep or cause to be kept in the said area any sugar-cane of a variety other than the varieties Uba, CH 64/21, P. O. J. 2878, and P. O. J. 2725 without a written permit from an officer appointed by the Minister of Agriculture.

5. Where under sub-section (2) of section *fifteen* of Act No. 11 of 1911, the Minister specially empowers an officer to take such steps as may be necessary for eradicating the disease on any premises, the said officer may require by fourteen days' notice the destruction of sugar-cane of any variety other than Uba and the destruction shall be carried out in the manner prescribed in the said notice.

Schedule

The following is the area restricted by Proclamation No. 290 of 1930 :—

The province of Natal and Zululand, excluding the Magisterial Districts of Ubombo and Ingwavuma.

* *No. 809* (*9th June, 1933*)

His Excellency the Governor-General has been pleased, in terms of section twenty-eight of the Agricultural Pests Act, No. 11 of 1911, to make the following amendments to the regulations published under Government Notice No. 2192 of 1930, as amended by Government Notice No. 1364 of 1931.

A. By the cancellation of the amendments published under Government Notice No. 1364 of 1931 and the substitution of the following new amendments.

B. By the cancellation of paragraph 2 and the substitution of the following new paragraph :—

(2) For the purpose of these regulations, the expressions " Variety Uba," "Variety CH 64/21," " Variety P. O. J. 2878 ", Variety P. O. J. 2725 ", "P. O. J. 2727 " " P. O. J. 2714 ", " Co 290 " and " Co 281 " shall be as defined in the Annexure to Proclamation No. 290 of 1930 as amended by Proclamation No. 119 of 1933.

C. By the addition of the words " P. O. J. 2727 ", " P. O. J. 2714 ", " Co 290 ", " Co 281 " after the words "P. O. J. 2725 " where they appear in paragraphs 3 and 4.

EXTRACT FROM UNION GOVERNMENT GAZETTE NO. 2299 OF 27TH SEPTEMBER 1935

* *No. 197, 1935.*

Under and by virtue of the powers in me vested by paragraph (*d*) of section *fourteen* of the Agricultural Pests Act, 1911 (Act No. 11 of 1911) as replaced by section two of the Agricultural Pests Amendment Act 1934 (Act No. 15 of 1934) I do hereby declare, proclaim and make known that Proclamation No. 290 of 1930, as amended by Proclamation No. 119 of 1933 is hereby further amended by the addition of the words " and also Co 301 " after the words " Co 281," where they appear in paragraph 2 of the Annexure to the said Proclamation No. 290 of 1930 as so amended.

The expression " Co 301 " is defined in the Annexure hereto.

God Save the King.

Given under my Hand and the Great Seal of the Union of South Africa at Pretoria this Twentieth day of September, One thousand Nine hundred and Thirty-five.

J. W. WESSELS,
Officer Administering the Government.

By Command of His Excellency the Officer Administering the Government-in-Council.
D. REITZ.

ANNEXURE

The expression " Co. 301 " means any sugar cane that has the characters of the leaf and stalk of the cane variety recognised by the Government Mycologist as Co 301 and that has been derived from the seed canes which were imported from the Imperial Sugar Cane Station, Coimbatore, South India, under the name of Co 301 by the issue of Plant Importation Permit No. D. 598 of the 18th October 1930.

REVIEW

Vernalization and phasic development of plants. *Bulletin No. 17 of the Imperial Bureau of Plant Genetics:* Herbage plants, and Occasional Publication of the Imperial Bureau of Plant Genetics for Crops other than Herbage: Joint Publication—Aberystwyth and Cambridge, Dec. 1935. (Price 10/-).

The idea of vernalization originated as a result of a new outlook on plant growth and development formulated by the Odessa school of plant physiologists headed by T. D. Lysenko. Until 1930, it was unknown to wider circles of agriculturists, though since that time it has assumed a very prominent place in agricultural literature and thought. The general principles of the theory are now widely familiar. These are roughly that growth and development, far from being identical as has been popularly supposed, are in reality two distinct and separate phenomena. Growth is regarded merely as increase in size and weight and such changes as flowering and reproduction are included under development. According to the new theory either of these two processes may proceed independently of the other, so that we may be faced with the extraordinary case of a plant which comes into flower without having grown or a plant which grows for ever without ever coming into flower: All that it is necessary to do it would seem, to produce such remarkable behaviour, is to treat the plant in such a way that one of the two processes is favoured to the exclusion of the other. If seeds of winter wheat are germinated in an ice box and subjected to suitable conditions of light, aeration, humidity and other factors, they can be sown in spring and will come into ear at the same time as if they had been sown in the ordinary way in autumn. Hence the term "vernalization," which is a Latinized equivalent of the Russian word "jarovizacija" coined by Lysenko to describe the phenomenon and which strictly means "transformation of winter forms into spring".

The matter was first brought to the attention of English-speaking readers by a bulletin issued in 1933 by the Imperial Bureaux of Plant Genetics. This early bulletin gave a complete outline of the method as it was described in Lysenko's first publications, and consisted mainly in the description of a practical method for the pre-sowing treatment of seed to obtain this accelerated development in a variety of crops. The method was rapidly seen to be of practical importance not merely in Russia but in all parts of the world. In view of the wide interest taken in the subject the Imperial Bureaux have continued to collect the literature relating to it. This has assumed such magnitude and the subject has become so much extended in its range that the whole matter has been reviewed afresh in a new and much enlarged bulletin covering some 150 pages.

This new bulletin describes in detail the results of the many experiments that have been carried out on all sorts of crops under every possible condition, both in the Soviet Union and in other countries all over the world. The results of vernalization of over 6,000 varieties of wheat and of 500,000 hectares of vernalized sowings, based on replies to a questionnaire received from 1,056 different Soviet farms are reported. The results have on the whole been satisfactory when the necessary conditions of technique have been observed and it would seem that the method may now be definitely regarded as a useful agricultural measure in countries where early ripening is a decisive factor in successful cultivation. For instance, spinach, sugar beet and even potatoes have by suitable treatment been grown at Hibiny in the arctic circle. Cereals have been induced to ripen a week or more earlier and by this means it has become possible to extend their cultivation to large tracts of country where this has been impossible hitherto because of drought or other unfavourable conditions. The main results of the Russian workers have now been confirmed in most countries where vernalization has been tried. In countries of more normal climatic conditions the method will probably not be of such wide application to the main crops but in the case of salad, vegetable and market garden crops a difference of a few days may prove of tremendous economic importance and a considerable future is foreseen in the application of vernalization to plants of this type. The Russian workers have even claimed to get increased yields from vernalized sowings but there seems to be some difference of opinion on this point and others have observed a depression in yield after vernalization.

At least half the bulletin is devoted to the physiological aspect of the phenomenon of vernalization. Lysenko's original conception has been extended to cover the whole range of plant development, in the form of the theory of Phasic Development. Development, says Lysenko, consists of a series of stages, each of which requires for its completion a definite combination of external conditions. The conditions for one phase may be different from those required for others, temperature being the decisive factor for the first stage and light for the second. Each stage must be completed before the succeeding one can be initiated but once any stage has been completed there is nothing to prevent the progress of the following one, provided the necessary external conditions are forthcoming. The method of vernalization therefore consists in the provision at as early a point in the life of the plants as possible of the requisite conditions for all the developmental stages leading up to reproduction, thus curtailing the long periods of time that normally occur between these successive stages. A freshly germinated seed has been found to be the most suitable material to work on, as this can be conveniently treated before sowing and then sown in the ordinary way.

Other Soviet botanists, though agreeing with Lysenko's general findings, disagree with many of his conclusions and have put forward theories of their own. The views of these other workers are presented in the bulletin side by side with those of Lysenko in a spirit of pure impartiality. In fact a careful study of this bulletin shews that it has dealt with the available information from all possible points of view, equally generous treatment being given to the practical application of the method in agriculture and horticulture and to its theoretical explanation. It will be of interest to plant physiologists, biochemists, plant breeders, teaching botanists and indeed to anyone concerned with modern trends of botanical and agricultural research. " The rapid development in scientific research is one of the outstanding features of the Twentieth Century," as Sir David Chadwick aptly remarks in his forward ; " Research workers need the earliest possible information of developments affecting their line of study but frequently find themselves baffled by its volume and by linguistic difficulties." To overcome these difficulties the Imperial Agricultural Bureaux were organized in 1929 and the bulletin under review affords an admirable example of how these Bureaux fulfil their function. The large majority of the articles with which the bulletin deals are written in Russian and would have remained entirely unknown to the rest of the world if they had not been made available in the present form. A total of over 200 separate articles are considered and reviewed in detail and large sections of the tabulated results of the authors are reproduced in a review of the work of the whole world on this controversial but entrancing new subject.

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NCAR—MB/11	No. 11.—Tables of Standard Errors of Mendelian Ratio, By Swarn Singh Purewal and P. Krishna Rao. Price As. 12 or 1*s*. 3*d*.
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